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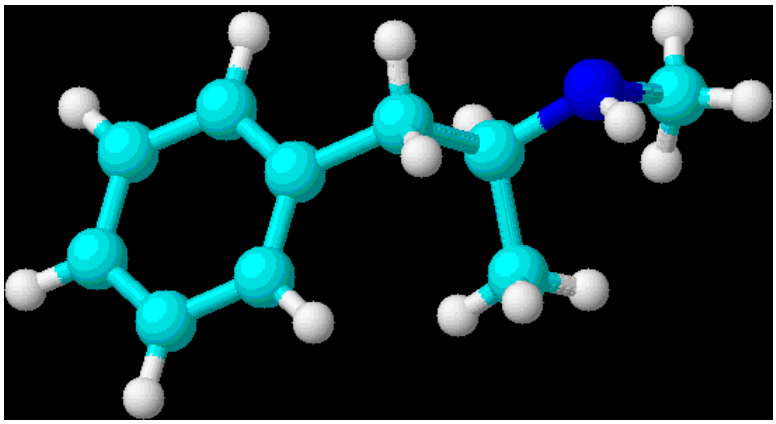

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Cover Art: "Ball and Stick" Model of Methamphetamine (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA)

* Letter to the Editor regarding: Mohammad Sarwar and John L. McDonald. A Rapid Extraction and GC/MS Methodology for the Identification of Psilocyn in Mushroom/Chocolate Concoctions. Microgram Journal 2003(3-4):177.

Sir:

While perusing the second issue of the *Journal* I noted an error in terminology in the article entitled: "A Rapid Extraction and GC/MS Methodology for the Identification of Psilocyn in Mushroom/Chocolate Concoctions" by Mohammad Sarwar and McDonald. The authors twice refer to the taxonomic categories of *strophariaceae*, *bolbitiaceae*, *coprinaceae*, and *cortinariaceae* as the four "species" of fungi which contain psilocyn and psilocybin. These categories are not species; they are families, as indicated by the suffix -"aceae". Each of these four families contains several genera, and each genus contains multiple species. The family *cortinariaceae*, for example, contains at least eight genera and well over a thousand different species. This is a small criticism in an otherwise excellent paper, and I congratulate the authors for their work.

Robert Parsons
Indian River Crime Laboratory, Fort Pierce, Florida

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Anise Oil as a Precursor for 2-Alkoxy-5-methoxybenzaldehydes

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ABSTRACT: Anethole, the principal component of anise oil, is occasionally utilized as a precursor to anisaldehyde, which in turn is used as a precursor in the illicit synthesis of 4-methoxyamphetamine and 4-methoxymethamphetamine. Anethole can also be utilized as a precursor for 2,5-dimethoxybenzaldehyde and 2-ethoxy-5-methoxybenzaldehyde. 2,5-Dimethoxybenzaldehyde is a precursor for designer dimethoxyphenylethylamines that are subject to abuse, such as 2C-B, DOB and DOI, while 2-ethoxy-5-methoxybenzaldehyde can be similarly used to synthesize some of the so-called "Tweetios" (methylene insertion analogs of the corresponding dimethoxy compounds). In these synthetic routes, anethole is first oxidized to anisaldehyde, which in turn is converted to 4-methoxyphenol via a Baeyer-Villiger reaction. The phenol is formylated via a Reimer-Tiemann reaction, and the resulting benzaldehyde can be methylated to give 2,5-dimethoxybenzaldehyde, or ethylated to give 2-ethoxy-5-methoxybenzaldehyde. The described procedures are of forensic and judicial interest.

KEYWORDS: Anise Oil, Anethole, Anisaldehyde, 4-Methoxyphenol, 2,5-Dimethoxybenzaldehyde, 2-Ethoxy-5-methoxybenzaldehyde, Forensic Chemistry

Introduction

Anise oil is the common trade name for the essential oils of two different plant species, *Pimpinella anisum* and *Illicium verum*. Most commercially available anise oil is derived from *Illicium verum* (also known as star anise), and is grown primarily in the Far East. Anise oil from *Pimpinella anisum* has a sweeter taste and a more agreeable odor, and is usually grown in Central Asia and the Mediterranean region.

The main component of anise oil is anethole, 4-methoxyphenyl-1-propene [**1**]. Both varieties of anise oil contain 80 - 90 % anethole (1a,b). The essential oil derived from fennel (*Foeniculum vulgare*) also has a high anethole content, usually 50 - 60 %. Anethole is industrially utilized as a precursor for 4-methoxyphenyl-2-propanone, a valuable chemical stock. We recently demonstrated that anethole had been used as the precursor for clandestinely prepared 4-methoxyamphetamine (PMA) or 4-methoxymethamphetamine (PMMA) through 4-methoxyphenyl-2-propanone (2). This synthetic route is analogous to the syntheses of the methylenedioxyamphetamines (MDA, MDMA, or MDEA) from 3,4-methylenedioxyphenyl-2-propanone, prepared from isosafrole.

During our study of the preparation of 4-methoxyamphetamine starting from anethole (2), we noted that 4-methoxyphenol [**3**] was formed during the performic acid oxidation of anethole in the synthesis of 4-methoxyphenyl-2-propanone (2). It was determined that 4-methoxyphenol was formed by the Baeyer-Villiger oxidation of anisaldehyde (4-methoxybenzaldehyde [**2**]), which was present in the reaction mixture as an impurity originating from the peracid oxidation of anethole. 4-Methoxyphenol is recovered in an industrial process using a similar per-oxidation procedure (3). Therefore, we decided to explore whether 4-methoxyphenol could be formed from anethole as the primary product (that is, not as a side-product). If so, this would represent a possible route for the preparation of several 2,5-dimethoxyphenethylamines and 2-ethoxy-5-methoxy-phenethylamines (see Figure 1).

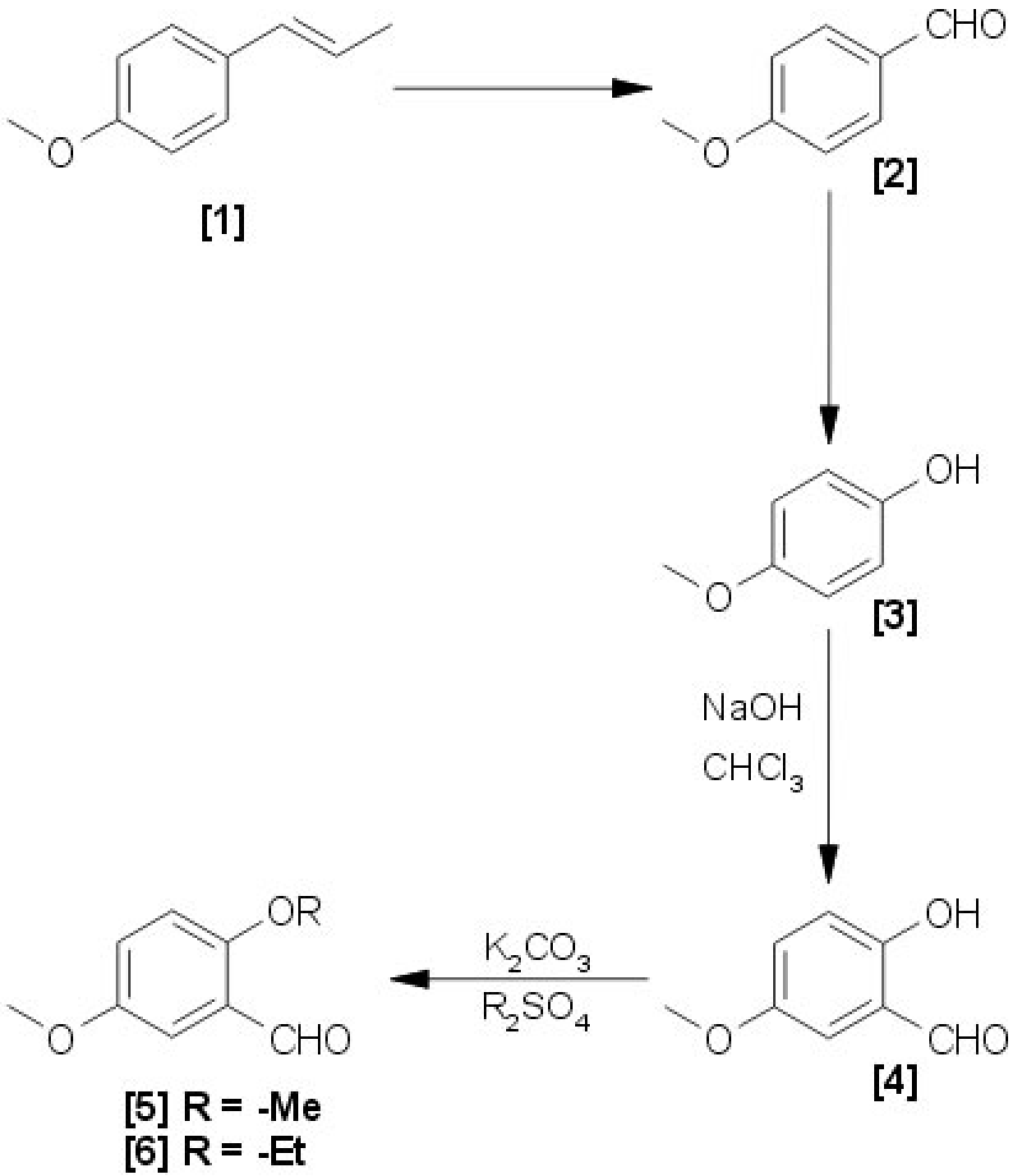

Figure 1: Anethole [**1**] is oxidized to anisaldehyde [**2**], which is subjected to a Baeyer-Villiger oxidation to give 4-methoxyphenol [**3**], which is subjected to a Reimer-Tiemann formylation to give 2-hydroxy-5-methoxybenzaldehyde [**4**]. Methylation gives 2,5-dimethoxybenzaldehyde [**5**], while ethylation gives 2-ethoxy-5-methoxybenzaldehyde [**6**]. Compounds **5** and **6** can be utilized as precursors for various 2,5-dimethoxylated phenethylamines or 2-ethoxylated-5-methoxylated phenethylamines. For details, see the Experimental Section.

Experimental

Chemicals and Reagents

All solvents used in this work were analytical grade and purchased from Acros Organics (Geel, Belgium). Anise oil was obtained from Taiga International NV (Breendonk-Puurs, Belgium), and originated from China (harvest year 2000) from *Illicium verum* (star anise). All other reagents were acquired from Merck (Darmstadt, Germany) or were synthesized from anethole (*vide infra*).

Instrumentation

Mass spectral analysis was performed on an Agilent 6890 Plus GC coupled to an Agilent 5973N MSD, and are presented in Figure 2. An HP-5-MS capillary column (30.0 m x 0.25 mm x 0.25 μm) was employed. Helium was the carrier gas, with a constant flow of 0.6 mL/min. The transfer line and ion source were operated at 280^{O} C and 230^{O} C, respectively. Mass spectra were recorded from 35 to 550 amu. The mass spectrometer was run in the Electron Impact (EI) mode with an ionization energy of 70 eV. A solvent delay of 4 min was applied. Oven temperature programming was as follows: 1 min at 50^{O} C, to 100^{O} C at 35^{O} C/min, to 270^{O} C at 10^{O} C/min. This temperature was maintained until the end of the programmed run (39.48 min). Injections were done split or splitless, depending on the nature of the sample.

Syntheses

Anisaldehyde (4-Methoxybenzaldehyde [**2**])

A freshly prepared and stirred solution of 30 mL concentrated sulfuric acid in 150 mL water was allowed to cool down to 30^{O} C, and anise oil (9.8 g) was added. A total of 25 g sodium bichromate was then added, at such a rate that the reaction temperature remained between 35 - 40^{O} C. The reaction mixture was extracted four times with toluene (75 mL each), and the combined organic phases were washed twice with 5 % NaOH (100 mL each), and once with water (100 mL). The organic phase was evaporated to about 20 mL, and anisaldehyde was then isolated as its bisulfite adduct. The yellow precipitate was washed with an EtOH/ether (1:1) mixture until the precipitate's color turned white (that is, similar to the bisulfite adduct generated from commercially available anisaldehyde). Setting the anisaldehyde free resulted in 4.9 g of a yellow oil with a pleasant odor. The mass spectrum was in agreement with an authentic sample. Anisaldehyde was the main product (95 % by GC/MS), but several minor impurities (not further identified in this report) were noted.

4-Methoxyphenol [**3**]

Performic acid was generated by mixing 23 g 30 % hydrogen peroxide with 19 mL 98 - 100 % formic acid and allowing it to react for 30 minutes. The resulting mixture was added to a stirred solution of 12 mL anisaldehyde in 200 mL dichloromethane, and refluxed for 24 h. The solvent was removed via rotavap, and the resulting residue was dissolved in a mixture of 200 mL NaOH (20 %) and 75 mL MeOH. This mixture was stirred for an additional hour, after which the MeOH was removed via vacuum distillation. The mixture was acidified with concentrated HCl to pH 1, and then extracted with dichloromethane (2 x 150 mL) . The combined extracts were dried over anhydrous Na_2SO_4, then evaporated via rotavap to give 10.0 g of a brownish oil which solidified upon standing. Further purification gave 4-methoxyphenol as a white crystalline product. The mass spectrum was in agreement with an authentic sample.

2-Hydroxy-5-methoxybenzaldehyde [**4**]

A 500 mL three-necked round bottom flask, equipped with reflux condenser, thermometer, and magnetic stirrer, was charged with 80 g NaOH and 100 mL water and stirred until dissolved. 30 g 4-methoxyphenol was then added to the still hot and stirring solution. Once the temperature dropped to 70^{O} C, 40 mL chloroform was added drop-wise over the course of 3.5 h, while the reaction temperature was maintained at 65 - 70^{O} C. During the reaction, yellow-green crystals formed on top of the mixture. When all of the chloroform was added, the reaction was continued for an additional hour, after which the mixture was acidified with chilled, 10 N H_2SO_4 to pH 2 - 3. A brown oil separated on top, and was isolated, and the residual aqueous phase was extracted with dichloromethane (2 x 100 mL). The combined organic phases were dried over anhydrous Na_2SO_4, and the solvent was removed via rotavap. The resulting oil was added to the previously isolated oily layer and

steam-distilled. The distillate (2.5 L) was extracted with dichloromethane, and the organic layer isolated and washed with chilled water. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed via rotavap. The residual yellow oil (2-hydroxy-5-methoxybenzaldehyde) weighed 23.8 g and was used in subsequent reactions without further purification.

2,5-Dimethoxybenzaldehyde [**5**]
A 250 mL round-bottomed flask was equipped with a reflux condenser, thermometer, and magnetic stirrer, and was charged with 14 g anhydrous potassium carbonate, 10 g 2-hydroxy-5-methoxybenzaldehyde, and 100 mL acetone, and the mixture was brought to reflux. Once the mixture was boiling, 11 g of dimethyl sulfate was added, and the reaction was refluxed. After 3.5 h, the mixture was cooled, filtered, and the solvent was removed. The residue was taken up in 100 mL of cold water, and the precipitated crystals were collected and recrystallized from water/EtOH (1:1), giving (after drying in vacuo) 8.3 g 2,5-dimethoxybenzaldehyde as faintly yellow tinted needle-shaped crystals (GC purity: 98 %+). The mass spectrum was in agreement with an authentic sample. 1H-NMR δ 3.799 (s, 5-OMe), 3.893 (s, 2-OMe), 6.942 (d, J = 9.1 Hz, 1H), 7.135 (dd, J = 3.3 & 9.1 Hz, 1H), 7.326 (d, J = 3.3 Hz, 1H), 10.44 (s, 1H). 13C-NMR δ 55.69, 56.06, 110.45, 113.33, 123.41, 124.98, 153.63, 156.76, 189.60 (CHO).

2-Ethoxy-5-methoxybenzaldehyde [**6**]
A setup similar to the one described for 2,5-dimethoxybenzaldehyde was charged with 7 g anhydrous potassium carbonate, 7 g 2-hydroxy-5-methoxybenzaldehyde, and 100 mL acetone, and the mixture was brought to reflux. Once the mixture was boiling, 5 mL diethyl sulfate was added, and the reaction was refluxed. After 3 h, the mixture was cooled, filtered, and the solvent was removed. The residue was taken up into 75 mL of cold water, and the precipitated crystals were collected and recrystallized from water/EtOH (1:1), yielding spectacularly long, needle-shaped crystals. Recrystallization from EtOH gave 5.9 g 2-ethoxy-5-methoxybenzaldehyde as faintly yellow tinted, polymorphic crystals (GC purity: 98 %+). 1H-NMR δ 1.447 (t, J = 7.1 Hz, 3H), 3.794 (s, 3H), 4.106 (q, J = 7.0 Hz, 2H), 6.925 (d, J = 9.1 Hz, 1H), 7.111 (dd, J = 3.3 & 9.1Hz, 1H), 7.317 (d, J = 3.3 Hz, 1H), 10.473 (s, 1H). 13C-NMR δ 14.57, 55.63, 64.74, 110.08, 114.48, 123.47, 125.14, 153.54, 155.21, 189.72 (CHO).

Results and Discussion

The synthesis of anisaldehyde from anethole can be accomplished in several ways, for instance by reaction with ozone (4), VO_5 (5), or HNO_3 (6,7). We opted for the well-known sodium bichromate mediated oxidation. The applied procedure is a minor adaptation of a method used in the fragrance industry (6). The aldehyde was purified via its bisulfite adduct instead of distillation. Isolation as the bisulfite adduct is - in this case - a facile and low-priced alternative for purification via distillation. In fact, in the early 20th century, the bisulfite adduct of anisaldehyde was commonly traded as *aubépine cristallisée* for use in the perfume industry (*aubépine* translates from French as “hawthorn” (8)).

The synthesis of 4-methoxyphenol from anisaldehyde can be performed via the Baeyer-Villiger oxidation reaction with hydrogen peroxide or a peracid (9). We utilized performic acid in this study, but other peracids such as peracetic acid (10) or *meta*-chloroperbenzoic acid (11) work equally well. Other possibilities include sodium perborate in glacial acetic acid (12-14) or hydrogen peroxide with boric acid (15). Yields usually range between 70 % and quantitative, depending on which method was used.

The Reimer-Tiemann formylation reaction (16) is not widely utilized. Generally, low yields, several side-reactions, and easy formation of intractable tars are problematic. However, submission of 4-methoxyphenol to a Reimer-Tiemann formylation gives acceptable yields and reasonable workups. The scientific literature contains many references concerning adaptations for the Reimer-Tiemann formylation of 4-methoxyphenol, with yields usually varying between 40 - 70 %. In our study, we opted for a previously reported procedure by Wynberg and Meijer (17). Generally, this method has several advantages over the Vilsmeier-Haack formylation

(another widely used formylation technique, but which gives poor yields in this case). Even an improved version of the Vilsmeier-Haack reaction still gave only 40 % 2,5-dimethoxybenzaldehyde after 48 h of refluxing (18).

The methylation of phenols to methoxybenzenes using dimethylsulfate is well-known. The use of dimethylsulfate requires care due to its toxicity, but it may be substituted for by less toxic and easier accessible chemicals, such as dimethyl carbonate (19).

The synthesized benzaldehydes can be used for the preparation of several “designer” phenylethylamines; 2,5-dimethoxybenzaldehyde can be applied in the synthesis of, e.g.: 2C-B (20a), 2C-I (20b), DOB (20c), DOC (20d), DOI (20e), and 2,5-DMA (20f). Other phenylethylamines can be synthesized using 1,4-dimethoxybenzene, e.g.: 2C-P (20g). 2-Ethoxy-5-methoxybenzaldehyde is a precursor for the so-called "Tweetios" (20h). Tweetios are methylene insertion analogues of the 2,5-dimethoxyphenylethylamines, where one or both methoxy groups are replaced by ethoxy groups. These compounds generally display less potency and shorter duration time than the 2,5-dimethoxy analogues, and so do not have high potential for clandestine synthesis. In this case, only the 2-ethoxy-5-methoxyphenylethylamines can be obtained.

Anethole is currently used in large quantities in the alcoholic beverage industry (e.g., for Ouzo or Ricard), and in oral hygiene products (21). It is also a valuable component in aromatherapy products. Due to this economic significance, it is unlikely that anise oil or anethole will become monitored or scheduled substances, despite their use in the illicit production of PMA and PMMA, their link with several PMA- and PMMA-related fatalities over the past few years (2,22), and/or their potential use towards synthesis of various designer phenethylamines. We are currently unaware of any examples of anise oil or anethole being used to produce designer phenethylamines, but still feel it is necessary to point this possibility, since it might become a preferred precursor in the future as chemical substance controls are gradually increased. It is also important to understand that the presence of anise oil or anethole in a clandestine laboratory does not automatically imply that the operator intended to synthesize PMA and/or PMMA; it is also possible that synthesis of a designer phenethylamine was intended. This can only be ascertained by a total review of all chemicals and notes present at the laboratory, and/or by operator interviews.

Acknowledgements

We are grateful to Prof. Dr. Roger Busson (REGA Institute, K. U. Leuven) for recording of the NMR spectra reported in this study.

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[Note: Patents were retrieved via the Espacenet website http://gb.espacenet.com]

[Figure 2 Follows.]

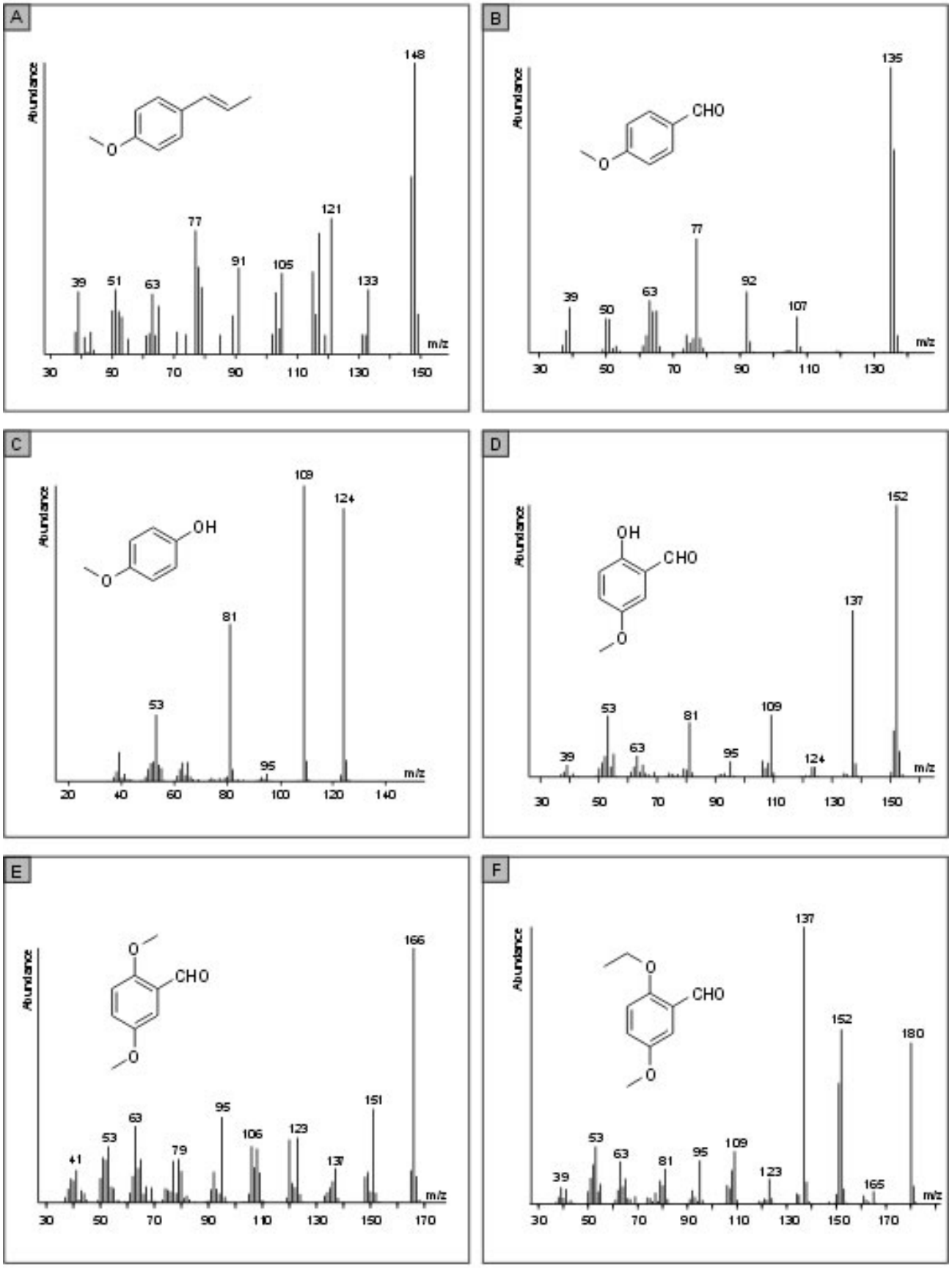

Figure 2: Mass Spectra of **1** - **6**.

Technical Note

Diltiazem HCl: An Analytical Profile

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ABSTRACT: Diltiazem, a potent vasodilator that is used in a wide variety of heart medications, was identified as an adulterant in several large shipments of illicit cocaine. Analytical data (gas chromatography, infrared spectroscopy, mass spectrometry, and proton nuclear magnetic resonance spectroscopy) are presented.

KEYWORDS: Diltiazem, Benzothiazepine, Calcium Channel Blocker, Vasodilator, Cocaine, Forensic Chemistry

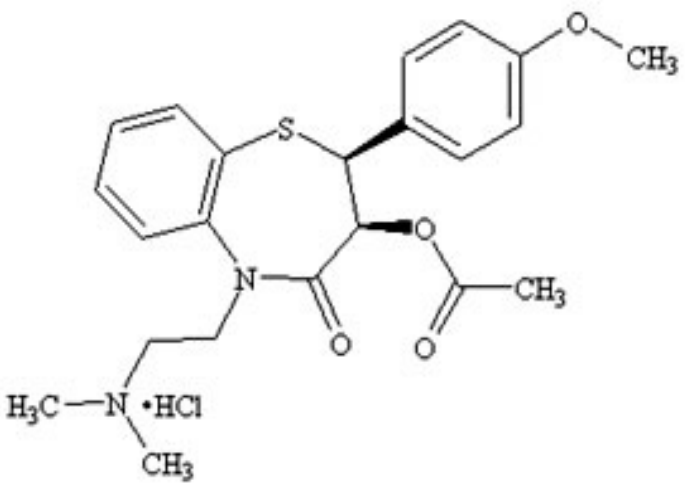

Figure 1: Structure of Diltiazem Hydrochloride

Introduction

This laboratory recently received samples from several multi-kilogram seizures of cocaine hydrochloride (ranging from 71 - 85 % cocaine HCl) containing varying amounts of diltiazem hydrochloride (8 - 20 %) (1,2). The full chemical name for diltiazem is (*2S-cis*)-3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (3) [Figure 1]. It is prescribed as a calcium channel blocker, and has potent vasodilating activity (4,5). This vasodilation is accomplished without additional oxygen consumption by the heart (6). These therapeutic properties have made diltiazem hydrochloride an important constituent in a myriad of heart medications which are widely prescribed for effects in combating angina, hypertension, and/or irregular heartbeats (7). Herein, we provide analytical data for diltiazem hydrochloride (8).

Experimental

Diltiazem: $C_{22}H_{26}N_2O_4S$ 414.53 amu

Source of Diltiazem
Sigma-Aldrich, Inc. (St. Louis, Missouri); Lot #123K0968, 99 %

Gas Chromatography

Instrument	Agilent 6890N with a flame ionization detector
Column	DB-1, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature	280° C
Oven Temperature	140° C for 1.5 min, 10° C/min to 280° C
Carrier Gas	Hydrogen at 1.1 mL/min, split ratio = 25:1

Utilizing the above experimental parameters, the retention time for diltiazem HCl is 17.64 minutes. The retention time relative to cocaine is 1.52. A screening run utilizing the above parameters will detect the presence of diltiazem and allow for correct quantitation parameters to be selected.

Infrared Spectroscopy

Instrument	Thermo-Nicolet Nexus 670
Number of Scans	32
Resolution	4.000
Wavenumber Range	4000 cm^{-1} to 400 cm^{-1}

Data was obtained by the use of an attenuated total reflectance (ATR) attachment on FTIR. The data was not ATR corrected [Figure 2]. In addition, spectral data was obtained with a KBr dispersion technique on FTIR [Figure 3]. The principal peaks are at 1680 cm^{-1} and 1250 cm^{-1}.

Mass Spectrometry

Instrument	Agilent 5973
Column	DB-1, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature	280° C
Oven Temperature	90° C for 2 min, 14° C/min to 300° C
Carrier Gas	Helium with split ratio = 25:1
Scan Range	34 - 550 amu

Electron impact mass spectrometry data shows a molecular ion at 414 amu and a base ion of 58 amu [Figure 4A]. When the ion abundance of this spectrum is enhanced 10x, the ions are more easily viewed [Figure 4B].

Nuclear Magnetic Resonance Spectroscopy
Analyses were performed on a Varian Mercury 400 MHz NMR. The sample was prepared at 22.4 mg/mL in deuterium oxide (D_2O) containing TSP (3-(trimethylsilyl)propionic-2,2,3,3-d4 acid, sodium salt) as the reference at 0 ppm and maleic acid as the internal standard. The maleic acid forms a singlet at 6.4 ppm. The proton spectrum of the standard was obtained with 8 scans using a 45 second delay, 90^o pulse, 5 second acquisition time, and oversampling of 4 [Figure 5].

Results and Discussion

The referenced exhibits appear to be the first identified to contain diltiazem. Based on cocaine signature analysis, it appears that the diltiazem was added to the cocaine during one of the final processing stages; either: A) the base was added to cocaine base and the two were co-precipitated as hydrochloride salts; or B) the hydrochloride was added to cocaine hydrochloride and physically mixed prior to pressing into kilogram bricks.

The purpose for adulterating illicit cocaine with such an unusual (and relatively expensive) compound is unclear. A (brief) review of several websites dedicated to drug abuse does not suggest any synergistic/desirable or pseudo-therapeutic effects to co-administration of diltiazem with cocaine. Therefore, it is most likely that it was used merely as a "cut of convenience".

Acknowledgements

The author wishes to thank Senior Research Chemist John F. Casale and Senior Forensic Chemist Pamela R. Smith (this laboratory) for their assistance. The author would also like to acknowledge Senior Research Chemist Patrick A. Hays (this laboratory) for his time and expertise in interpreting the NMR spectrum of diltiazem hydrochloride.

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[Figures 2 through 5 follow on the next three pages.]

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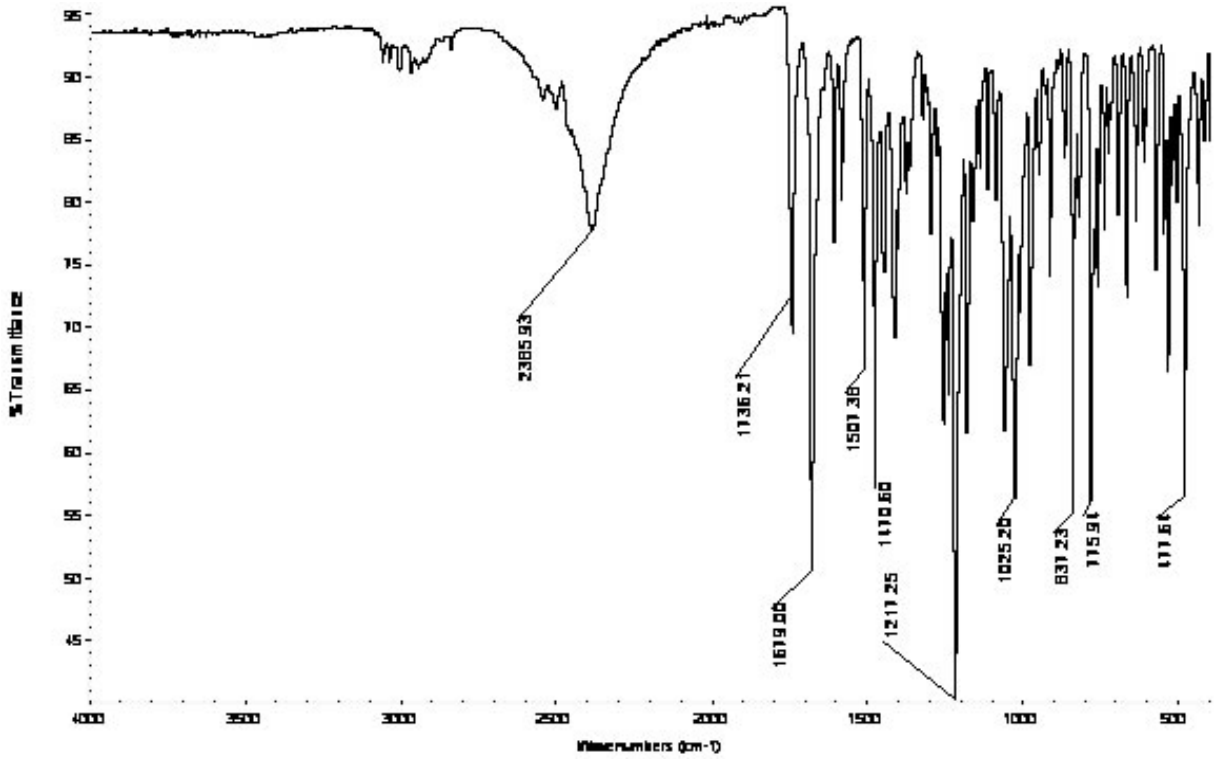

Figure 2: Uncorrected FTIR-ATR Spectrum of Diltiazem Hydrochloride

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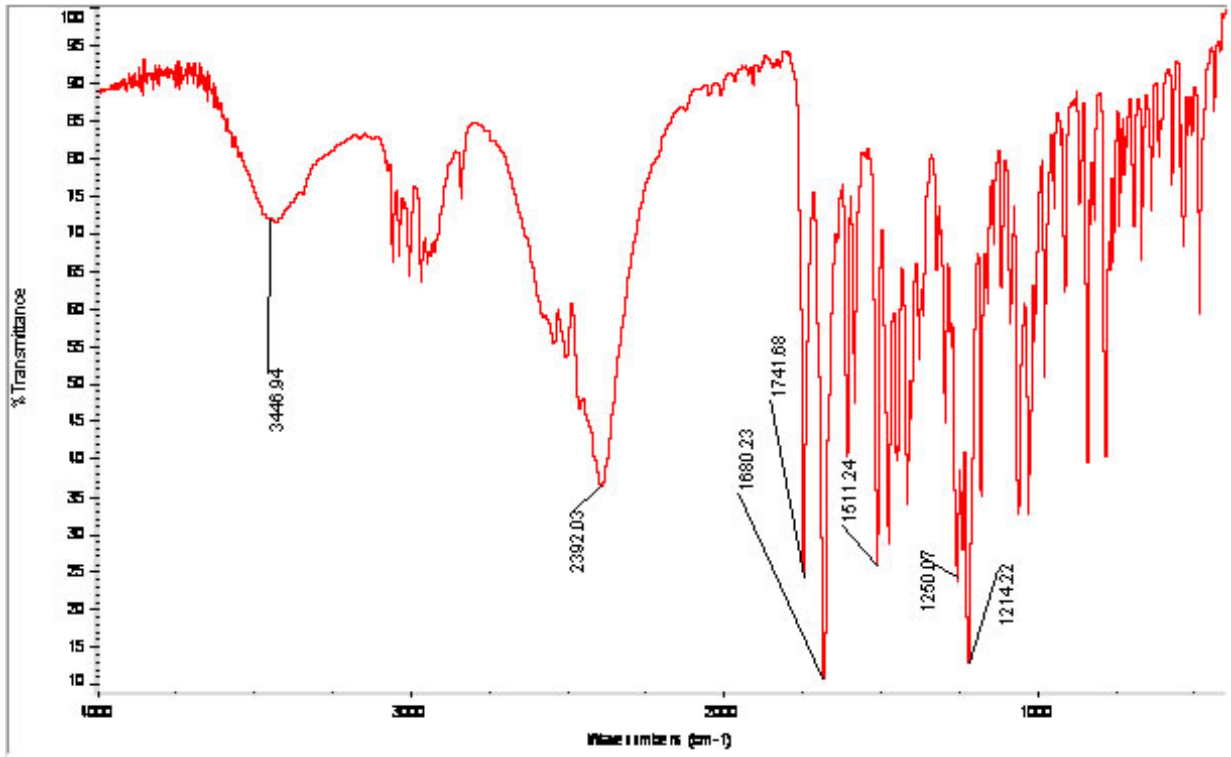

Figure 3: KBr Dispersion FTIR Spectrum of Diltiazem Hydrochloride

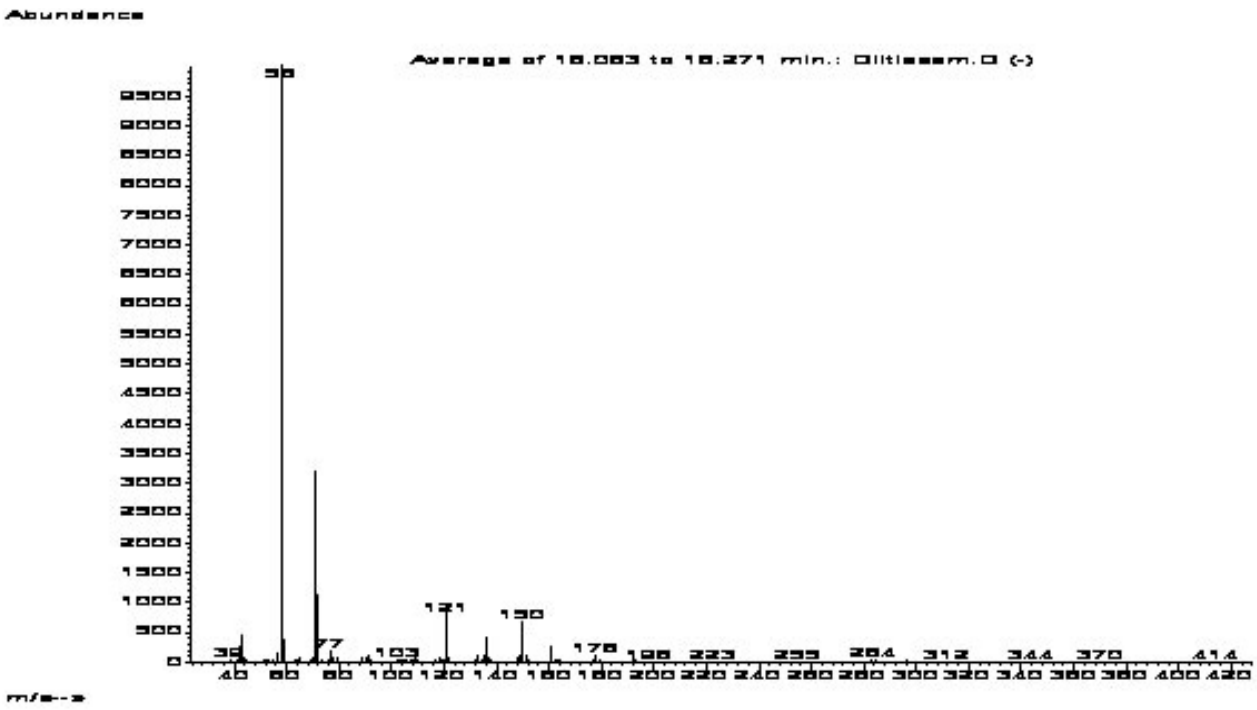

Figure 4a: Electron Impact Mass Spectrum of Diltiazem

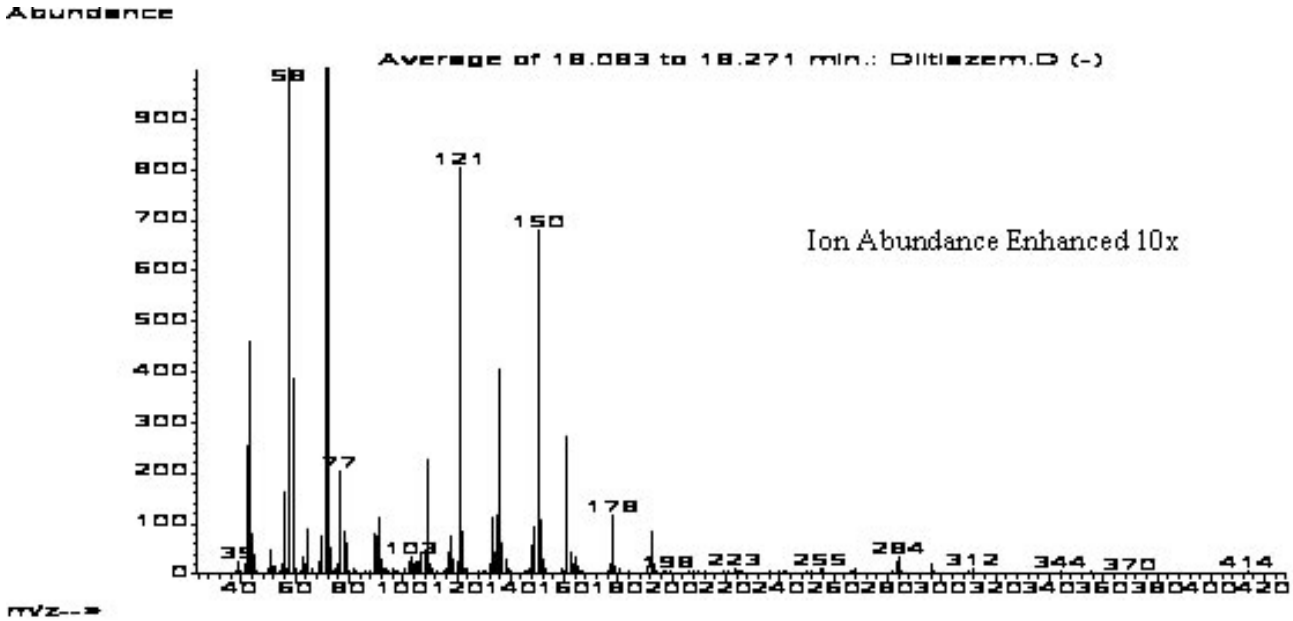

Figure 4b: Electron Impact Mass Spectrum of Diltiazem (Enhanced 10x)

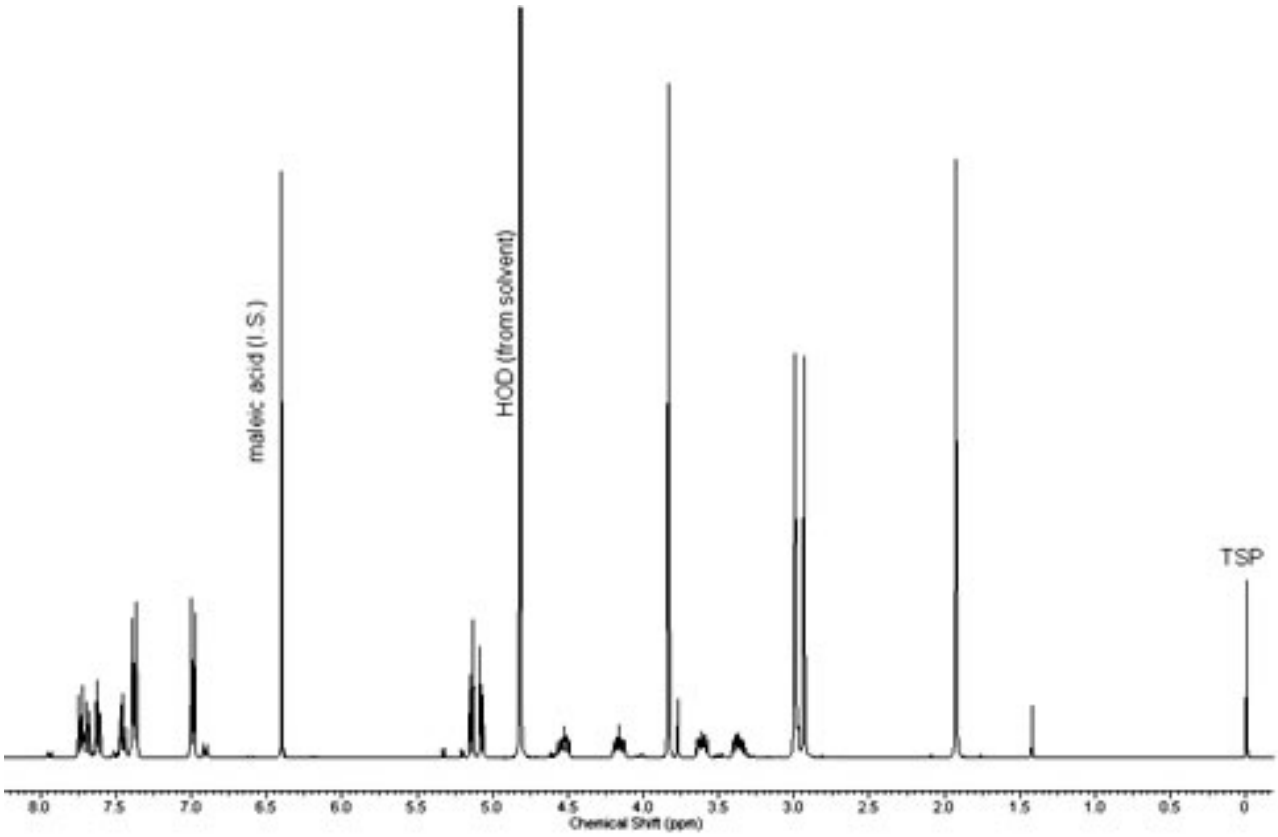

Figure 5: 400 MHz Proton NMR Spectrum of Diltiazem Hydrochloride in D_2O

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Technical Note

Hydroxyzine: An Analytical Profile

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ABSTRACT: Hydroxyzine, an anxiolytic and antihistamine, was identified as an adulterant in several large shipments of illicit cocaine. Analytical data (gas chromatography, infrared spectroscopy, mass spectrometry, and proton nuclear magnetic resonance spectroscopy) are presented.

KEYWORDS: Hydroxyzine, Anxiolytic, Antihistamine, Cocaine, Forensic Chemistry

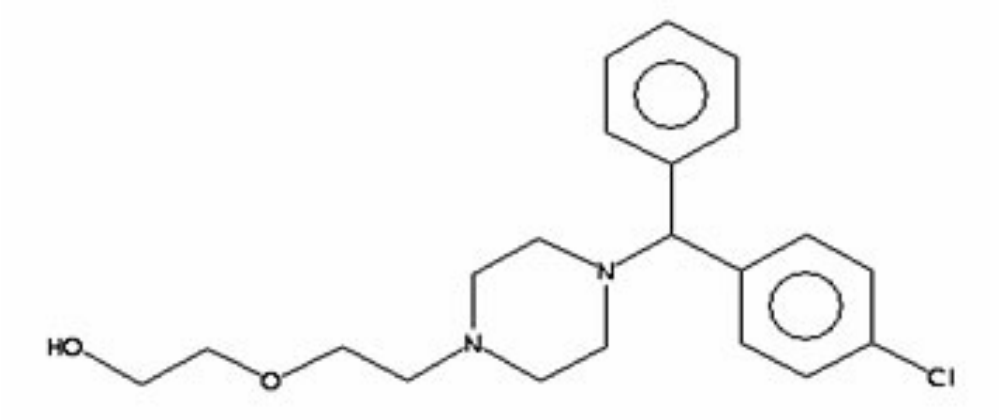

Figure 1: Structure of Hydroxyzine

Introduction

This laboratory recently received samples from several multi-kilogram seizures of cocaine hydrochloride containing varying amounts of hydroxyzine (2 - 20 %) (1). The full chemical name for hydroxyzine is 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]ethanol. Hydroxyzine is marketed as an anxiolytic (used in treatment of anxiety), antihistamine (allergy), and as a mild tranquilizer (2,3). It is sold in tablet form. Federal law restricts this drug to prescription use only. Herein, we provide analytical data for hydroxyzine (4).

Experimental

Hydroxyzine: $C_{21}H_{27}ClN_2O_2$ 374.91 amu

Source
Sigma, Inc. (Atlanta, Georgia); Lot #083K0522

Gas Chromatography

Instrument	Agilent 6890N with a flame ionization detector
Column	HP-1, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature	280° C
Oven Temperature	250° C Isothermal
Carrier Gas	Hydrogen at 1.1 mL/min, split ratio = 25:1

Utilizing the above experimental parameters, the retention time for hydroxyzine is 10.63 minutes. The retention time relative to cocaine is 2.85.

Infrared Spectroscopy
Infrared spectra were obtained on a Nexus 670 FT-IR equipped with a single bounce attenuated total reflectance (ATR) accessory (Figure 2).

Mass Spectrometry

Instrument	Agilent 6890 interfaced with an Agilent 5973 MSD
Column	DB-1, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature	280° C
Oven Temperature	90° C for 2 min, 14° C/min to 300° C
Carrier Gas	Helium with split ratio = 25:1
Scan Range	34 - 550 amu
Electron Ionization	70 eV

The Total Ion Chromatogram (TIC) for hydroxyzine is shown in Figure 3. The fragmentation pattern shows a molecular ion at m/z 374 and a base peak of m/z 201 (Figure 4). Hydroxyzine may also be further characterized by GC/MS after derivatization. Hydroxyzine (5 mg) was reacted with a mixture of 250 μL of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) in 250 μL of chloroform at 80° C for 30 minutes. The mass spectrum of the resulting trimethylsilyl (TMS) derivative gives a molecule ion at m/z 446 (Figure 5).

Nuclear Magnetic Resonance Spectroscopy
One dimensional proton NMR analyses were performed on a Varian Mercury 400 MHz NMR using a 5 mm Nalorac Indirect Detection probe. The sample was prepared at 10-30 mg/mL in deuterium oxide (D_2O) containing TSP (3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt) as the reference at 0 ppm (Aldrich Chemical Co., Milwaukee, Wisconsin). Maleic acid was used as the internal (quantitation) standard. The proton spectrum of the standard was obtained with 8 scans using a 45 second delay, 90^o pulse, 5 second acquisition time, and oversampling of 4 (Figure 6).

Results and Discussion

The referenced exhibits appear to be the first identified to contain hydroxyzine. Based on cocaine signature analysis, it appears that the hydroxyzine was added to the cocaine hydrochloride and physically mixed prior to pressing into kilogram bricks.

The purpose for adulterating illicit cocaine with such an unusual (and relatively expensive) compound is unclear. A (brief) review of several websites dedicated to drug abuse does not suggest any synergistic/desirable or

pseudo-therapeutic effects to co-administration of hydroxyzine with cocaine. Therefore, it is most likely that it was used merely as a "cut of convenience".

Acknowledgements

The author wishes to thank Senior Research Chemist John F. Casale and Forensic Chemist Heidi L. Wojno (this laboratory) for their assistance. The author would also like to acknowledge Senior Research Chemist Patrick A. Hays (this laboratory) for his time and expertise in interpreting the NMR spectrum of hydroxyzine.

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4. See also: Terry Mills III and J. Conrad Roberson, Instrumental Data for Drug Analysis, 2nd Ed., Vol. 2, pp.1182 - 1183; Elsevier, New York: 1987 (includes UV, MS, NMR, and FTIR data).

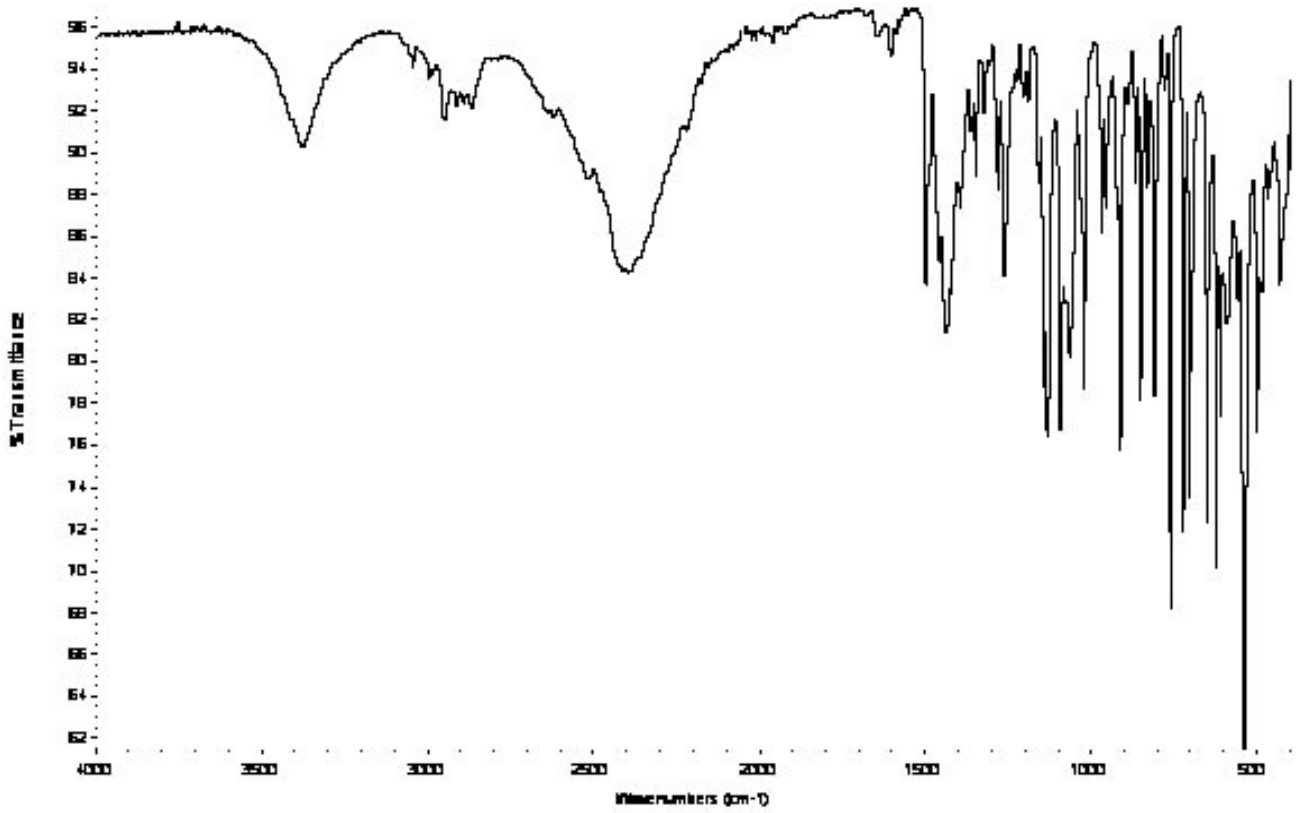

Figure 2: FTIR-ATR Spectrum of Hydroxyzine Hydrochloride

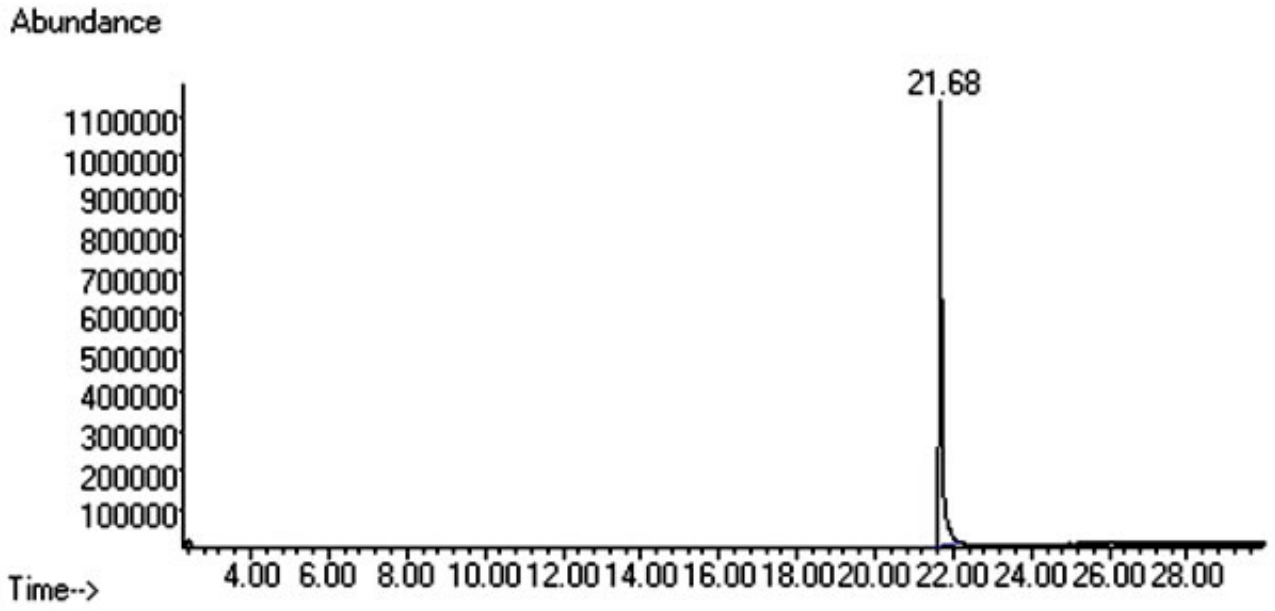

Figure 3: Total Ion Chromatogram of Hydroxyzine

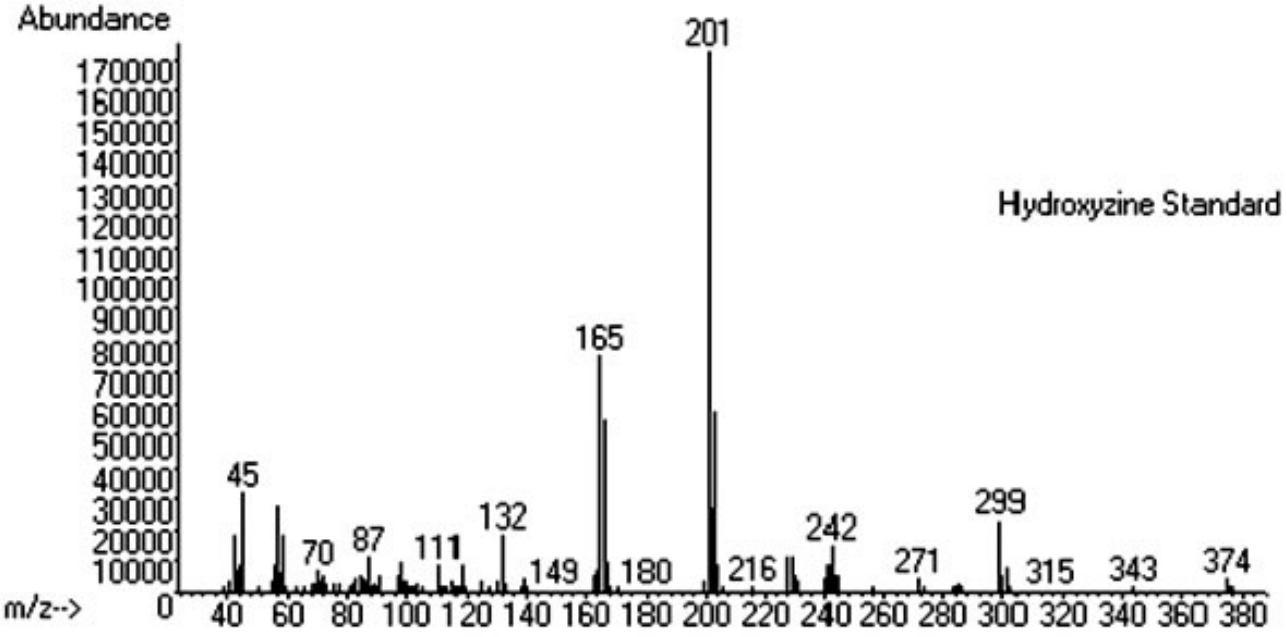

Figure 4: Electron Ionization Mass Spectrum of Hydroxyzine

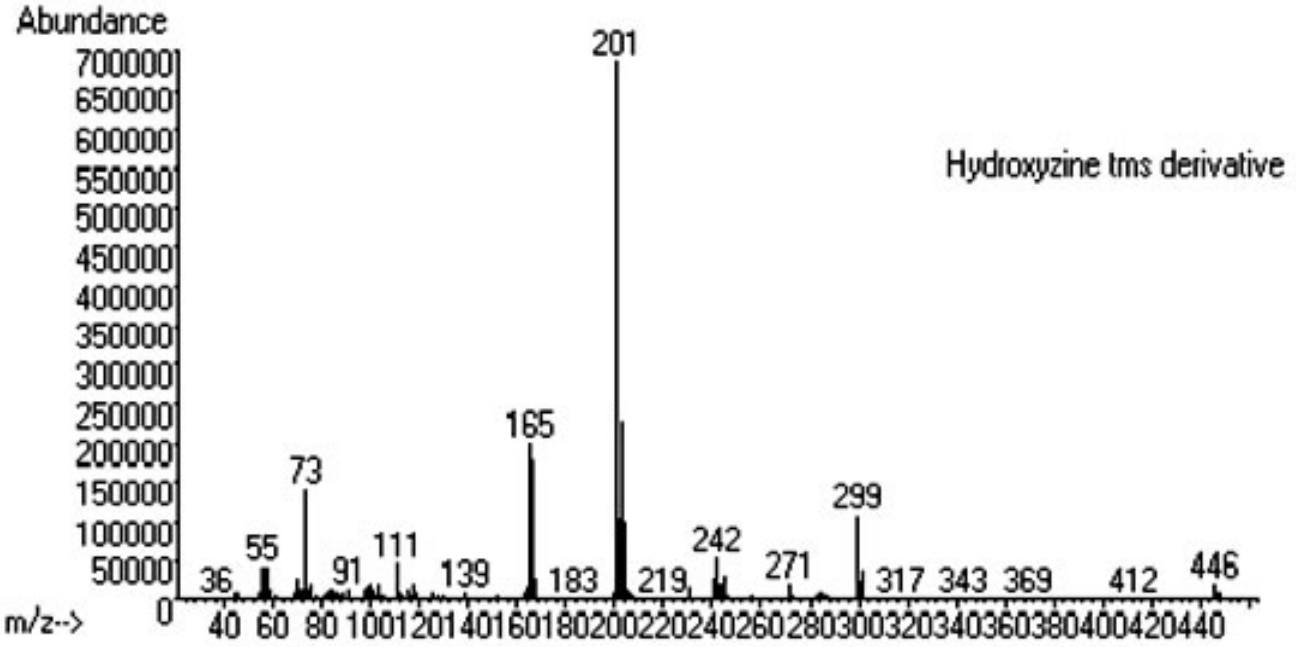

Figure 5: Electron Ionization Mass Spectrum of the Trimethylsilyl Derivative of Hydroxyzine

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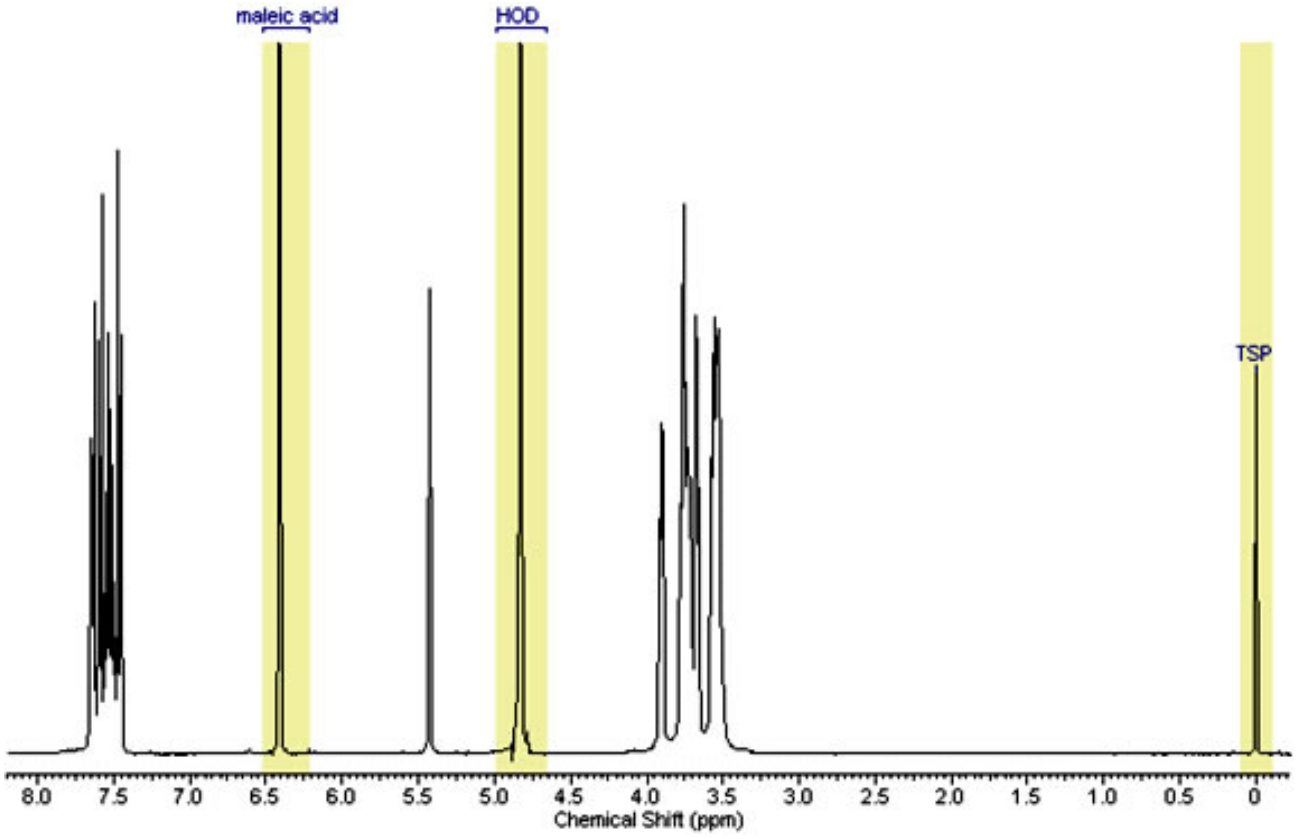

Figure 6: 400 MHz Proton NMR Spectrum of Hydroxyzine in D_2O

Technical Note

Mass Spectra of Select Benzyl- and Phenyl- Piperazine Designer Drugs

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ABSTRACT: The mass spectra of five piperazine designer drugs (N-benzylpiperazine, 1-(3,4-methylenedioxy-benzyl)piperazine, 1-(3-trifluoromethylphenyl)piperazine, 1-(3-chlorophenyl)piperazine, and 1-(4-methoxy-phenyl)piperazine) and their trimethylsilyl derivatives are presented.

KEYWORDS: Benzylpiperazines, Phenylpiperazines, Designer Drugs, Mass Spectrometry, Trimethylsilylation, Forensic Chemistry

Designer drugs of the benzyl- or phenyl- piperazine type, i.e., benzylpiperazine (BZP) itself, its methylenedioxy analogue 1-(3,4-methylenedioxybenzyl)piperazine (MDBP), 1-(3-trifluoromethylphenyl)piperazine (TFMPP), 1-(3-chlorophenyl)piperazine (mCPP), and 1-(4-methoxyphenyl)piperazine (MeOPP), recently have gained popularity and notoriety. Seizures have been made throughout the world (1-9), and a few fatalities have been reported (10-11). The increasing abuse of piperazines in the United States resulted in the temporary placement of BZP and TFMPP into Schedule I of the Controlled Substances Act (12). BZP was permanently scheduled in March, 2004 (13); however, TFMPP is currently not controlled in the United States.

Recently, many GC/MS studies on the metabolites of piperazines (i.e., from biological fluids) and/or their acetyl or heptafluorobutyryl derivatives have been published (14-24). However, most forensic drug laboratories perform GC/MS on the underivatized or trimethylsilylated derivatives of amine drugs. In Figures 1 and 2, the structures, electron-ionization mass spectra, and gas chromatographic retention indices (recorded on an Agilent GC-MSD 5972, HP-1 column, 12 m x 0.2 mm I.D., 100-310^{O} C, 30^{O} C/minute (25)) of the target piperazines and their trimethylsilyl derivatives are displayed. Additional data for these and several related piperazines will be published elsewhere (26-27).

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[Editor's Notes: * All issues of *Microgram* prior to January 2003 are law enforcement restricted. Selected references on the analysis of various piperazines were presented in *Microgram Bulletin* 2004;37(4):76.]

Figure 1: Structures, electron-ionization mass spectra, and gas chromatographic retention indices of underivatized piperazine-derived designer drugs.

Figure 2: Structures, electron-ionization mass spectra, and gas chromatographic retention indices of trimethylsilylated piperazine-derived designer drugs.

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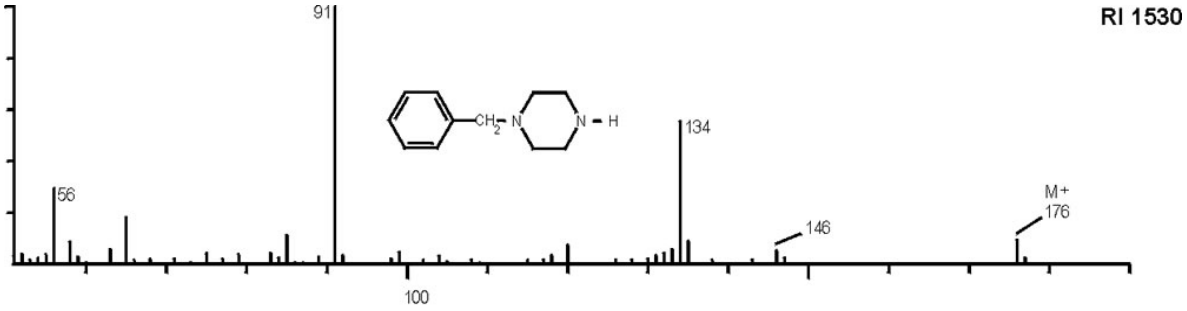
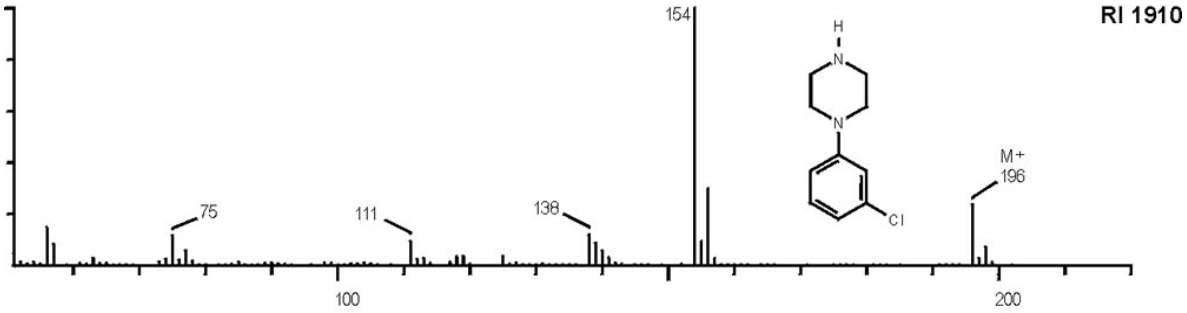
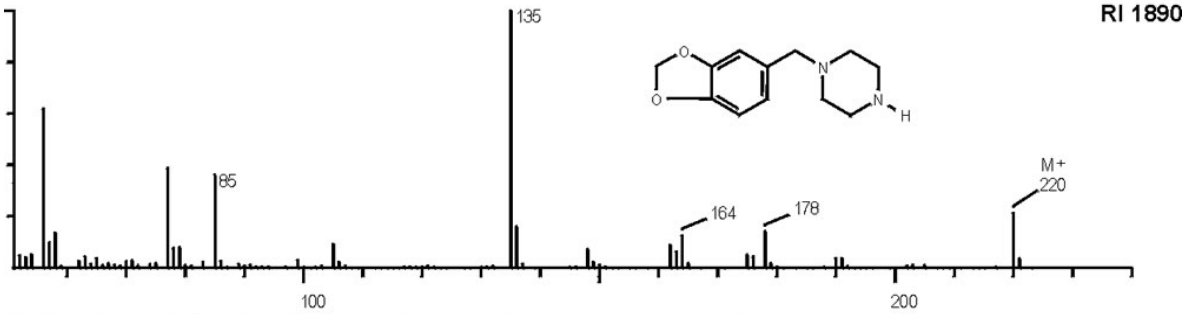


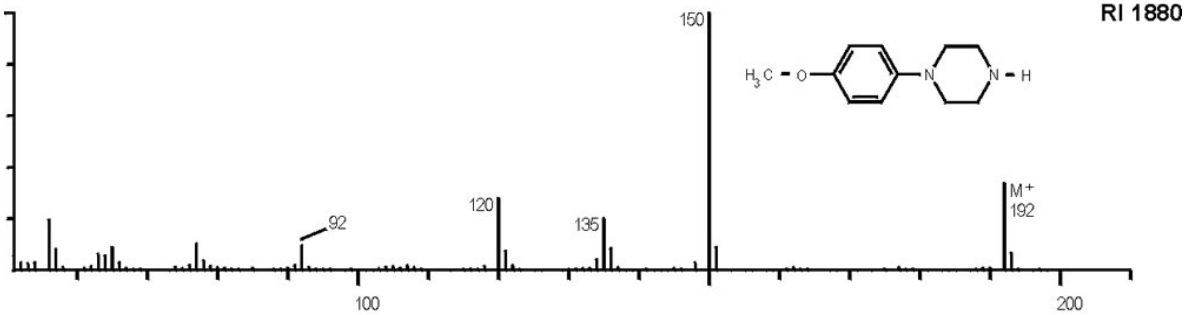
Benzylpiperazine BZP



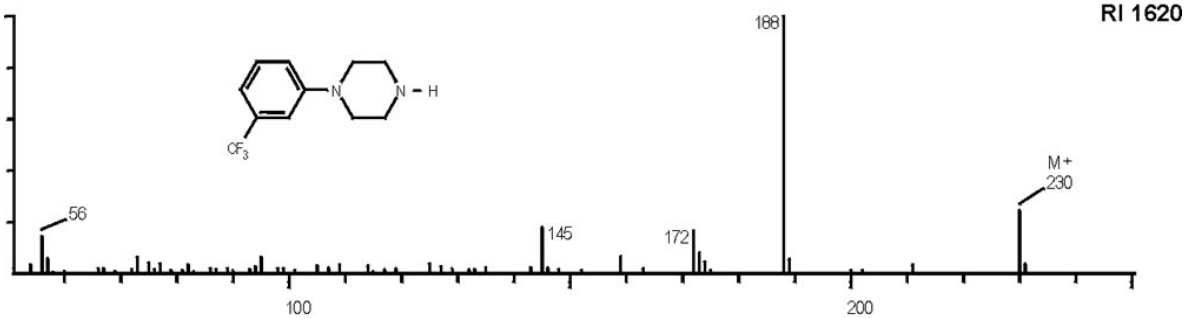
mCPP m-Chlorophenylpiperazine Nefazodone-M (N-desalkyl-) Trazodone-M (N-desalkyl-)



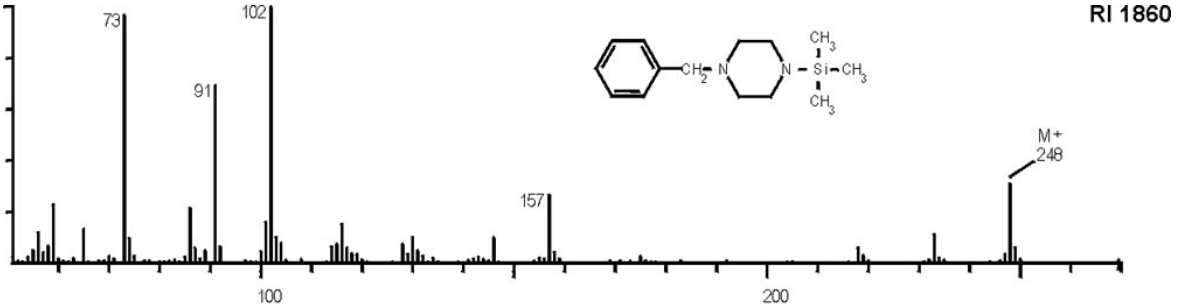
MDBP Fipexide-M/artifcat (MDBP) Methylenedioxybenzylpiperazine Piperonylpiperazine



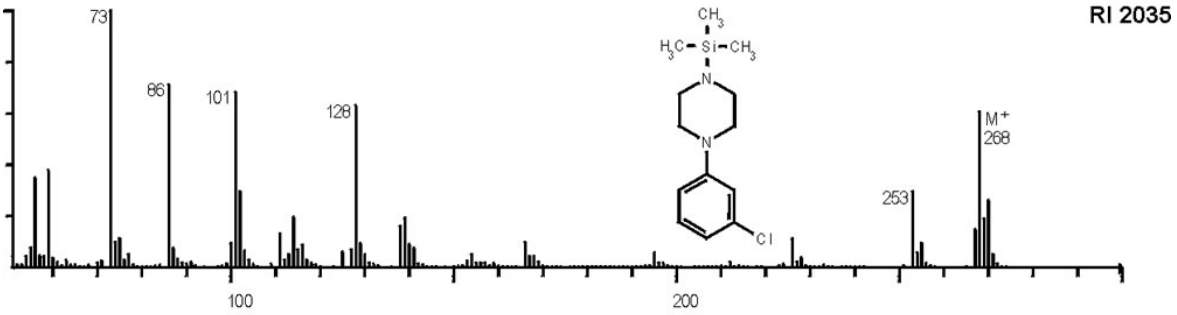
MeOPP 4-Methoxypiperazine



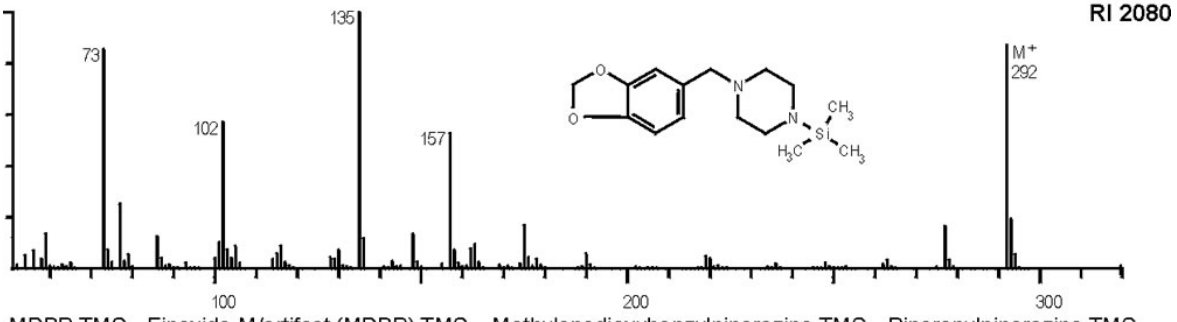
TFMPP Trifluoromethylphenylpiperazine



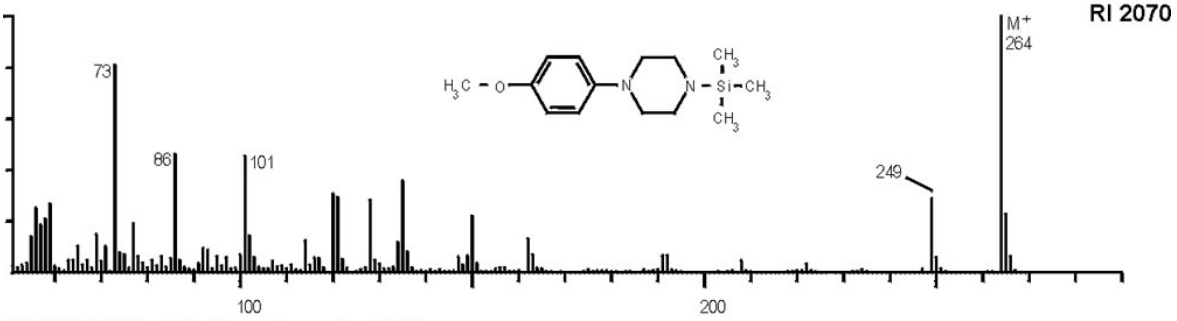
Benzylpiperazine TMS BZP TMS



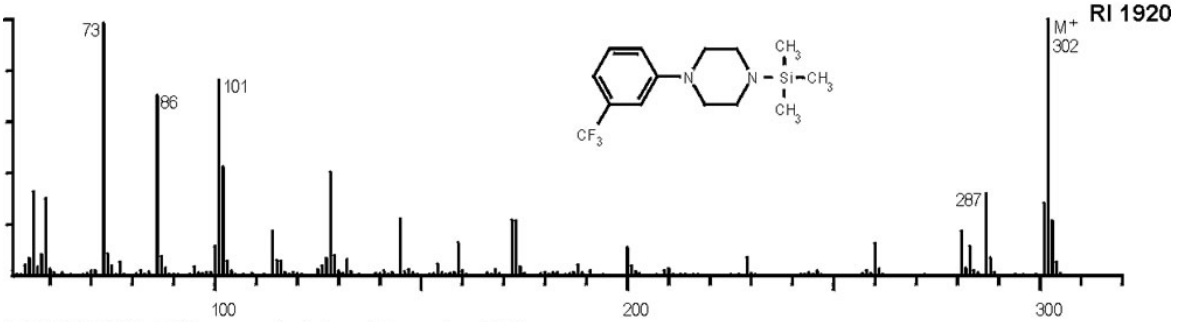
mCPP TMS m-Chlorophenylpiperazine TMS Nefazodone-M (N-desalkyl-) TMS Trazodone-M (N-desalkyl-) TMS



MDBP TMS Fipexide-M/artifcat (MDBP) TMS Methylenedioxybenzylpiperazine TMS Piperonylpiperazine TMS



MeOPP TMS 4-Methoxypiperazine TMS



TFMPP TMS Trifluoromethylphenylpiperazine TMS

The Quantitation of Nimetazepam in Erimin-5 Tablets and Powders by Reverse-Phase HPLC

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ABSTRACT: The sedative-hypnotic nimetazepam in "Erimin 5" tablets and powders was quantitated by reverse phase HPLC. The selectivity, precision, and accuracy of the procedure are presented.

KEYWORDS: Nimetazepam, Erimin-5, Benzodiazepines, HPLC, Forensic Chemistry

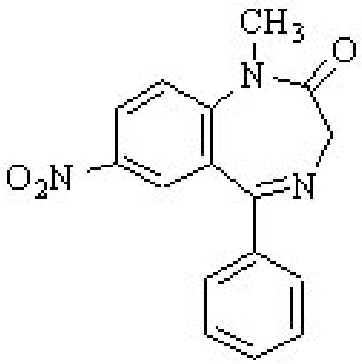


Figure 1: Structure of Nimetazepam

Introduction

Since its appearance in illicit drug markets in Malaysia in the mid-1980's, the benzodiazepine nimetazepam (Figure 1) has become the most commonly abused sedative in the country (midazolam and triazolam are the (distant) second and third most abused sedatives). The popularity of nimetazepam is due in part to its wide availability and relatively low price on the local black markets, and in part due to its long activity. Most of the abusers are believed to be heroin addicts, who use it as a substitute for heroin when its availability is low. More recently, however, nimetazepam has also been used as a sedative by methamphetamine abusers to help them sleep after binging (in fact, the rise in nimetazepam abuse roughly parallels the rise in methamphetamine abuse in Malaysia). The illicit use of nimetazepam is continuing to increase, as shown by the number and size of seizures made over the past few years. For example, a seizure of 310,000 tablets was made in June 2002 at a residence near the capital city (Kuala Lumpur). Tablet submissions to the Central Laboratory have been in the hundreds of thousands for each of the three years 2002 - 2004. Similar abuse of nimetazepam has been reported in neighboring countries.

The two primary forms of nimetazepam encountered in Malaysia are a commercial product (Erimin-5 tablets in blister packs (see Photos 1 - 2)) or Erimin-5 counterfeits, and an orange colored powder that appears to be either finely crushed tablets or the tablet mixture prior to tableting. Commercially prepared tablets nominally weigh

about 170 mg and contain about 5 mg of nimetazepam each. However, as noted above, many of the Erimin-5 tablets submitted to the Narcotics Section appear to actually be counterfeit products that contain nimetazepam and/or various other benzodiazepines, notably diazepam and nitrazepam, in varying quantities.

Nimetazepam was added to the Malaysian Dangerous Drugs Act 1952 in May, 2001 and is currently the only benzodiazepine controlled in Malaysia. The analysis of nimetazepam by a variety of techniques has been previously reported (1-4), including by CE and CEC (5-7), Color Testing (8), FTIR (9), GC (10-12), HPLC and HPLC/MS (13-18), TLC (17,19), and UV/Vis (20). Herein, we report the quantitation of nimetazepam in seized tablets and powders with reverse-phase HPLC, using an external standard method.

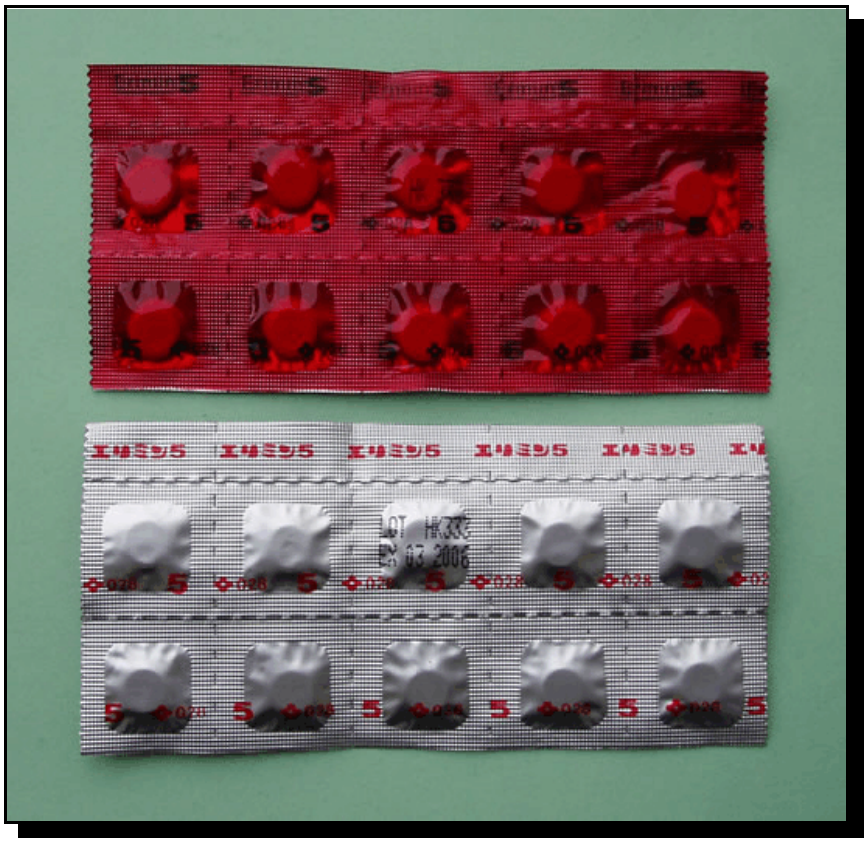


Photo 2 - Front and Back Views of a Erimin-5 Blister Pack (Note: This is a Suspected Counterfeit)

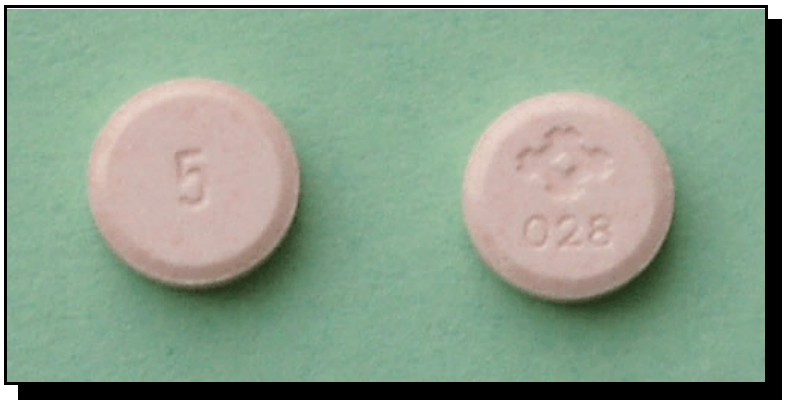


Photo 2 - Closeup of an Erimin-5 Tablet (Front and Reverse)

Experimental

Chemicals

HPLC grade methanol and chloroform were purchased from Merck, while AR grade orthophosphoric acid (84 %) was purchased from Ajax (Australia). Nimetazepam (free-base) standard of 100 % purity was kindly provided free of charge by Sumitomo Chemical Company (Tokyo, Japan). The following benzodiazepines (as free bases) were obtained from the United Nations Drug Control Programme (UNDCP) in Vienna (Austria): Nitrazepam, bromazepam, tetrazepam, flunitrazepam, oxazepam, lorazepam, clorazepate dipotassium (salt), diazepam, flurazepam, and medazepam. Unfortunately, midazolam and triazolam standards were unavailable, and so were not run.

Instrumentation

A Hewlett Packard Series 1050 HPLC was used with the following parameters:

Column :	C-18, 5 μm particle size, 15 cm x 4.6 mm i.d. (from Alltech).
Detector :	UV at 265 nm.
Mobile phase:	Methanol:Water (50:65). The pH was adjusted to 4.0 with orthophosphoric acid (to a mixture of 500 mL of methanol and 650 mL of water was added one drop of orthophosphoric acid) (21).
Column temperature:	25º C (ambient temperature).
Flow rate :	1.5 mL/minute.
Average Pressure:	155 bar.
Injection:	20 μL by Rheodyne loop injector.
Attenuation:	4 (Integrator).

Standard Solutions for Linearity Study and Calibration

Standard solutions containing 0.020, 0.040, 0.080, 0.120, 0.160, 0.200 and 0.240 mg/mL of nimetazepam were prepared in a mixture of methanol/chloroform (5:1) (note that the chloroform was added to better solubulize the tablet materials, and had no adverse effects on the chromatography).

Quantitative Analysis of Samples

About 70 – 100 mg of homogenized tablet material was accurately weighed into a 25 mL volumetric flask and made up to volume with a mixture of methanol/chloroform (5:1). The sample solution was ultrasonicated for 5 minutes and filtered through a 0.45 μm filter before injected onto the column. Quantitation was by external standard and with reference to the peak area of the 0.120 mg/mL nimetazepam standard.

Procedure for Standard Addition Method

(i) 350.80 mg of tablet material was weighed into a 100 mL volumetric flask, made up to volume with methanol/chloroform (5:1), and ultrasonicated for 5 minutes. (ii) 10 mL of the solution in (i) (i.e., equivalent to 35.08 mg of tablet material) was pipetted into each of five 25 mL volumetric flasks. (iii) The following aliquots of nimetazepam standard stock solution (1.00 mg/mL) were pipetted into the solutions in (ii): 0, 1, 2, 3, and 4 mL. (iv) The solutions were made up to volume (i.e., 25 mL) with methanol/chloroform (5:1). (v) The solutions were filtered through a 0.45 μm filter and injected into the HPLC. (vi) A graph of area versus concentration of nimetazepam (mg/mL) was plotted using Excel and the native nimetazepam content calculated.

Results and Discussion

Selectivity

Identification of benzodiazepines is accomplished in this laboratory by GC/MS. However, GC and GC/MS are problematic for quantitation of nimetazepam and some related benzodiazepines due to thermal degradation at injector port temperatures, and so HPLC was selected for quantitation. Because of the wide diversity of chemical structures and solubility characteristics among the benzodiazepines, no single HPLC method will separate all of

them. The specificity of the method presented herein was defined in terms of the benzodiazepines typically found in Malaysia. The identities and retention times of these benzodiazepines using the presented methodology are presented in Table 1.

Table 1: Retention Times of Benzodiazepines (HPLC)

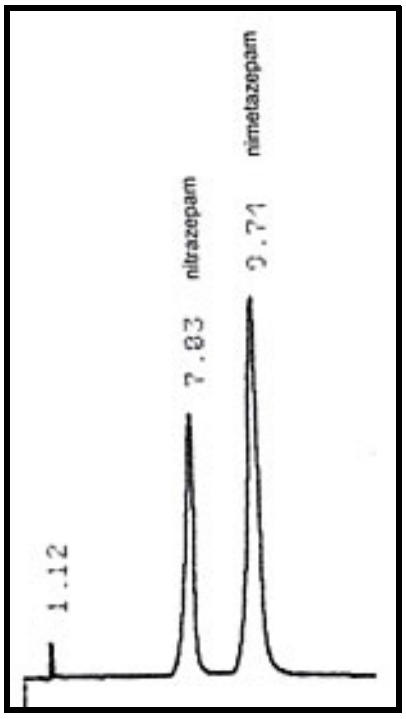
Benzodiazepine	Retention Time (min)
Nitrazepam	6.91
Bromazepam	7.62
Tetrazepam	7.80
Flunitrazepam	8.54
Oxazepam	8.55
Lorazepam	8.93
Nimetazepam	9.94
Clorazepate dipotassium	17.07
Diazepam	26.63
Flurazepam	NE
Medazepam	NE

NE: Did not elute within 30 minutes.

Of the selected benzodiazepines, flunitrazepam, oxazepam, and lorazepam elute closest to nimetazepam, and give partially overlapping peaks. Thus, the presented HPLC method is not appropriate for samples containing these compounds. Fortunately, however, experience has shown that these three benzodiazepines are very rarely present in tablets or powders containing nimetazepam. A few samples of "Erimin-5" tablets have been found to contain diazepam instead of nimetazepam; however, diazepam elutes much later than nimetazepam. A typical HPLC chromatogram of a mixture of nitrazepam and nimetazepam is displayed in Figure 2.

Figure 2: HPLC of Nitrazepam and Nimetazepam

Note: Slight variations in Retention Times between Table 1 and Figure 2 are due to natural variations over time; the order of elution was found to be consistent from run to run.



Calibration Curve and Linearity

The calibration graph (Figure 3) for the analysis was found to be linear from 0.020 mg/mL to 0.240 mg/mL. From linear regression analysis, the correlation coefficient was better than 0.99, and the percent difference between the known concentration and the predicted concentration from the regression equation was less than 5 %. In routine analyses a single point calibration was used.

Precision

The precision of the method was assessed by 10 replicate analyses of a homogenized sample of "Erimin 5" tablets. Injections were all made in triplicate and quantitation was

against the 0.120 mg/mL standard. The mean content of nimetazepam was found to be 3.1 % with a relative standard deviation of 4.4 %.

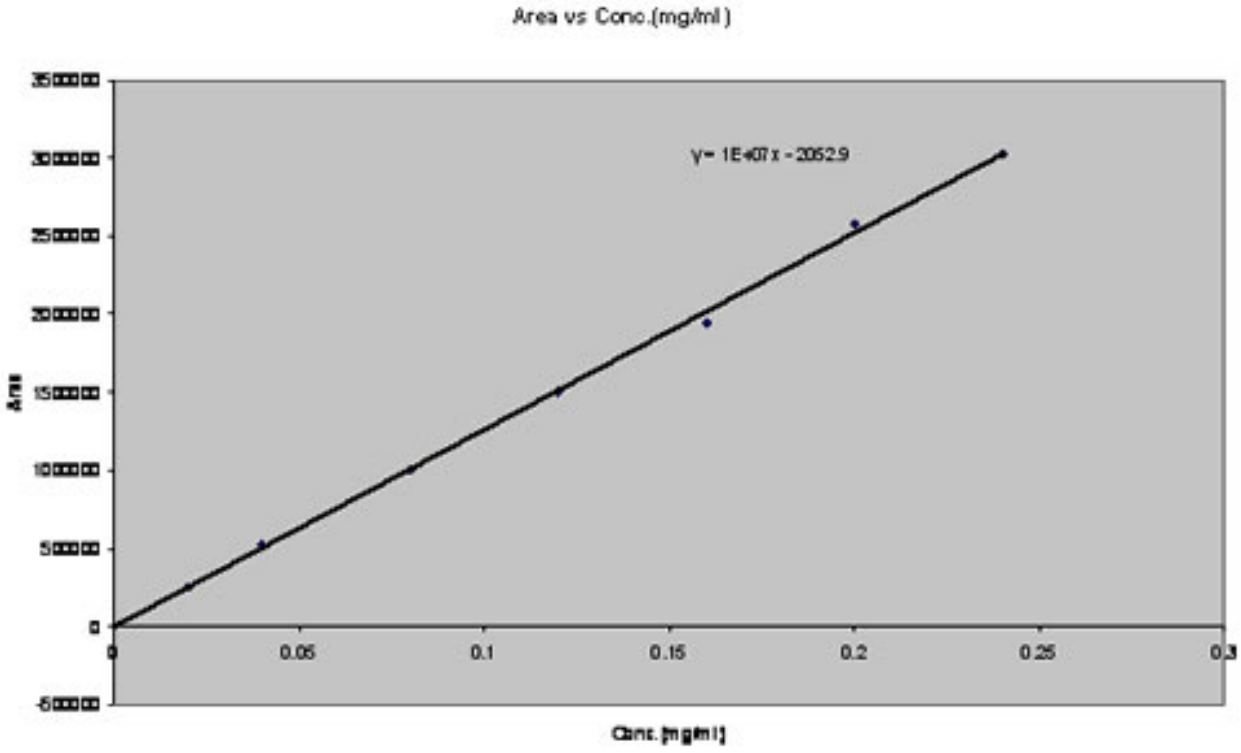


Figure 3: Calibration Curve of Nimetazepam

Accuracy

The accuracy of the method was assessed by analyzing two laboratory prepared mixtures, and re-analysis of the sample which was used for the precision study by the method of standard addition.

Analysis of Laboratory Prepared Mixtures

Owing to the limited supply of pure nimetazepam reference standards, only two synthetic mixtures were prepared, simulating 5 mg/tablet and 3 mg/tablet. Both the samples were prepared in lactose and contained 3.5 % and 1.7 % of nimetazepam, respectively. Replicate analyses (n = 7) of these two mixtures were made and the results assessed using the Student t-statistic:

$$t = | \bar{x} - \mu | \frac{\sqrt{n}}{s}$$

where $\bar{x}$ = sample mean (experimental value)
μ = true value (theoretical value)
n = no. replicate (weighings)
s = standard deviation

For both samples it was found that the t value did not exceed the critical value derived by statistical analysis, showing that there was no proven evidence of difference between the experimental value and the theoretical value at 95 % confidence level.

Standard Addition Method

The same nimetazepam tablet material which was used in the precision study was re-analyzed using the standard addition method. From the standard addition calibration graph (Figure 4) the amount of nimetazepam was found to be 3.1 %. This agreement with the precision study mean value shows that there is no interference from the tablet excipient materials, and thus to some extent shows that the method is accurate.

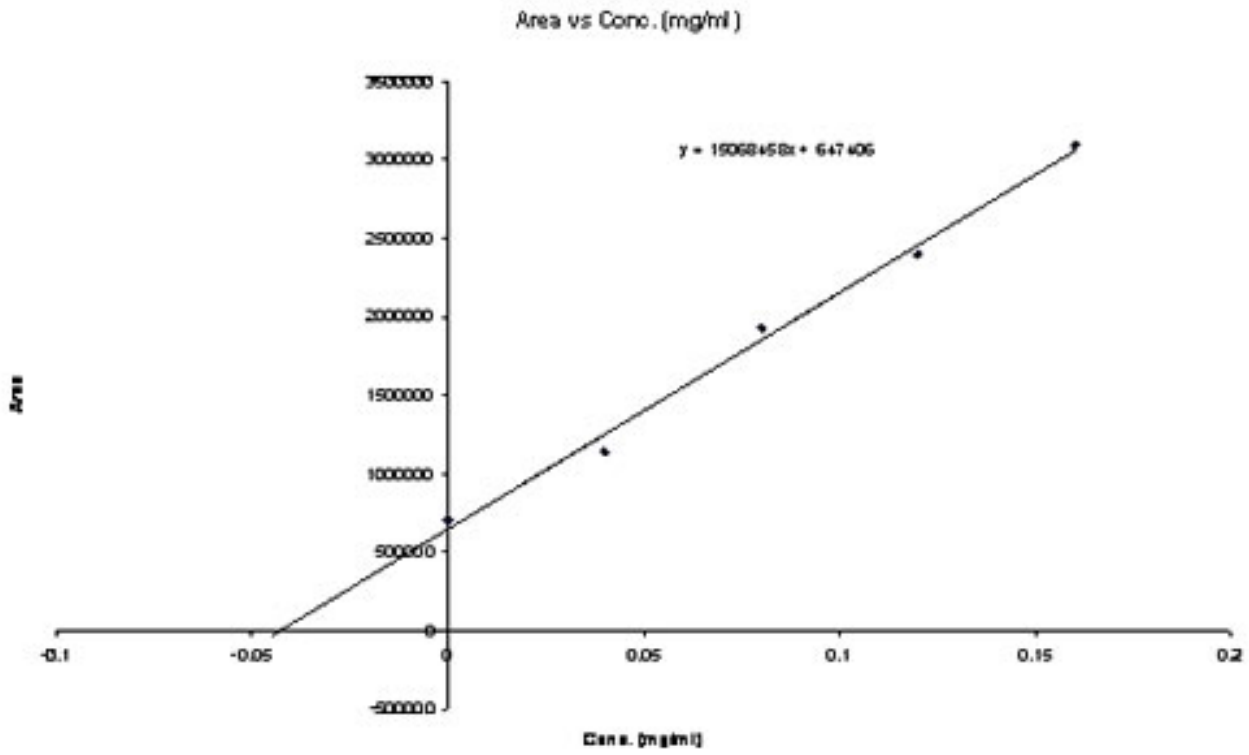


Figure 4: Standard Addition Calibration Curve

Acknowledgments

The authors would like to thank Dr. Yoji Sakito, Manager, Corporate Planning & Coordination Office, Sumitomo Chemical Company Ltd, Tokyo, Japan for the nimetazepam reference standard used in this study.

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Technical Note

Improvised Explosive Device Disguised as a Smoking Pipe

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ABSTRACT: A homemade pipe that was initially suspected to be intended for smoking controlled substances was instead found to actually be an improvised explosive device fabricated from match heads and air gun pellets.

KEYWORDS: Improvised Explosive Device, IED, Drug Paraphernalia, Pipe, Forensic Chemistry

Introduction

With the exception of needles, most drug paraphernalia items do not present a threat to the investigating officers or the forensic scientists examining the evidence. However, in a multi-exhibit case submitted to this laboratory in September, 2004 an improvised explosive device was discovered during examination and analysis. The case included six exhibits: One charred metal pipe (a typical "crack pipe"), one charred glass pipe (another typical "crack pipe"), three handmade aluminum foil pipes with charred residue (typical marijuana pipes), and one apparent pipe-like device wrapped with silver colored duct tape (see Photo 1). The seizures were made in a suburb of Pittsburg, Pennsylvania.

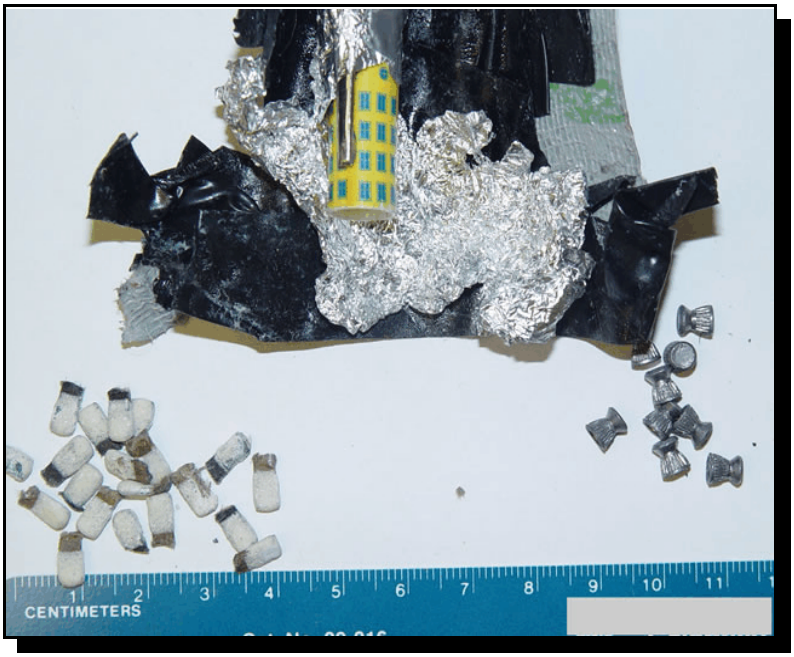

Photo 1

Experimental

The two presumed "crack pipes" (the metal and glass pipes) were each rinsed with chloroform, and the resulting solutions screened by thin layer chromatography (TLC), using "Analtech" Silica Gel plates 250 µm thickness. A reference standard of cocaine base was also spotted on the plate. The remainder of each chloroform rinse solution was evaporated to dryness, and the resulting residue was analyzed using Fourier Transform Infrared Spectrometry (FTIR), using a Perkin Elmer Spectrum One with an ATR attachment.

The three presumed marijuana pipes (the handmade aluminum foil pipes) were unrolled and found to contain charred residues. This material was subjected to a morphological examination under a stereoscope with a 10x magnification. The Duquenois-Levine test was also performed. Finally, the pipes were each rinsed with chloroform, and the resulting solutions screened by TLC against a known reference standard of marijuana.

The last pipe was first examined visually and stereoscopically (see Photo 1). It measured 8 cm in length and 1.5 cm in diameter, and was wrappeded with silver colored duct tape . As the layers of duct tape were unwrapped, an inner layer of black electrical tape was observed, and then a layer of aluminum foil. Inside of the foil, ten metal pellets were observed surrounding a plastic pen cap. The total net weight of the ten pellets was 76.9 grains. The pen cap was plastic, with a blue checked pattern on a yellow background. A standard metal shirt clip was observed on the exterior side of the pen cap. Twenty apparent match heads were found inside the pen cap. It appeared that the base of each match had been torn off, so that only the ignitable tip remained. The suspected match heads were examined using FTIR and diffuse reflectance.

Results and Discussion

The metal and glass pipes both tested positive for cocaine base. However, all three of the aluminum foil pipes tested negative for controlled substances. The final pipe was determined to actually be an IED. Upon close examination of the metal pellets, they were consistent in appearance with lead air gun pellets. The metal pellets had an hourglass shape with one end open characterized by rib marks around the outside; this is referred to as a "gereffelt" type skirt. The other end was filled and flat; this is referred to as a "wad cutter" type head design. The white material on the suspected match heads was consistent with potassium chlorate, one of the reactive ingredients used in the heads of commercially prepared matches. Although the exact function of this device is not known, detonation upon handling, disassembly, or analysis by law enforcement personnel seems unlikely. For this device to activate, an external force or an open flame would have to be introduced to ignite the match heads. The release of energy caused by the burning match heads would presumably cause an explosive effect, fragmenting the pen cap and ejecting the pellets contained within the tape. Speculating, it appears that the intended target would be an unsuspecting user.

[Editor's Notes: Based on a brief review, references dedicated to IED's do not include any devices of this exact description. However, there are a variety of similar, match head type IED's, most of which would be considered to be "booby-trap" devices designed to detonate upon handling. Specific references are withheld in accordance with *Journal* policy.]

* *

Identification and Determination of Carisoprodol in Tablets by Liquid Chromatography/Mass Spectrometry

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ABSTRACT: A method for the identification and determination of carisoprodol in tablet dosage form is described. Tablets were ground and carisoprodol was extracted using acetonitrile with sonication. Extracts were filtered and further dilutions were made with water. The identification of carisoprodol was accomplished using a single quadrapole mass spectrometer coupled to a liquid chromatograph with an electrospray source and positive ion detection. For determining the carisoprodol content, selected ion monitoring of the molecular ion was used. A 5 µm 2.1 x 150 mm Zorbax SB-C18 column and a mobile phase of 35 % acetonitrile (0.1 % formic acid) and 65 % water (0.1 % formic acid) at a flow rate of 0.4 mL/minute provided adequate retention. The calibration curve generated during this analysis was linear between 0.5 and 40 µg/mL for carisoprodol with a correlation coefficient, $r \geq 0.9996$. Spikes of tablets gave an average recovery of 93 % for carisoprodol.

KEYWORDS: Carisoprodol, Meprobamate, Liquid Chromatography/Mass Spectrometry, ESI-LC/MS, Forensic Chemistry

Introduction

Carisoprodol is a centrally acting muscle relaxant, with analgesic properties[1], making it a popular drug of abuse. Carisoprodol is widely available for purchase on the internet with or without a prescription. It is also available in combination with other analgesics, such as aspirin and codeine. Within the body, carisoprodol is metabolized to meprobamate (Figure 1), an anti-anxiety agent prescribed primarily to treat anxiety, tension, and associated muscle spasms. Meprobamate's onset and duration of action are similar to the intermediate-acting barbiturates; however, therapeutic doses produce less sedation and toxicity than barbiturates. This conversion may account for some of the properties associated with carisoprodol. These barbiturate-mimicking properties likely contribute to its abuse[2].

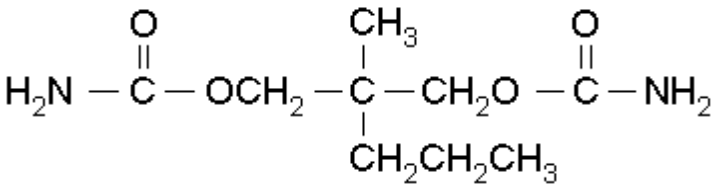

Figure 1: Meprobamate

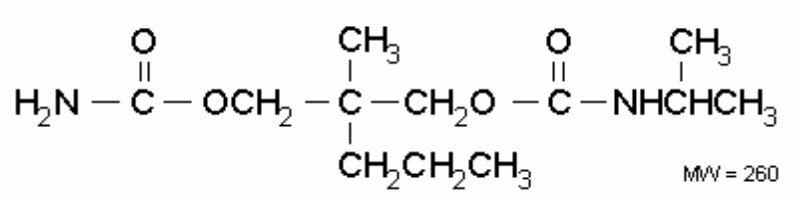

Figure 2: Carisoprodol

The structure of carisoprodol (Figure 2) is such that it does not have a UV chromophore with significant absorbance. Therefore, the USP Assay method for Carisoprodol Tablets employs a liquid chromatograph equipped with a refractive index detector[3]. Our laboratory does not currently have an operational refractive index detector, and numerous literature searches resulted in few references to carisoprodol analysis. We performed analysis of the carisoprodol tablets using a liquid chromatographic (LC) separation similar to that in the USP, but with mass selective (MS) detection. This paper describes the mass spectral identification of carisoprodol and the determination of the carisoprodol content of five individual tablets, each containing an unknown amount of carisoprodol.

Experimental

Apparatus

1. LC-MS System: Agilent (Agilent Technologies, Atlanta, GA) 1100 series LC-MSD with an electrospray source. Chemstation G1701AA version A.09.01 was used for data acquisition and processing.
2. Analytical column: Zorbax SB-C18, 2.1 x 150 mm, 5 mm (Agilent Technologies, Part # 883700-922).
3. Syringe filters: 25 mm diameter 0.45 µm Nylon syringe filters (National Scientific, Catalog #F2500-1), or equivalent.

Materials

1. Mobile phase: 35 % acetonitrile (0.1 % formic acid) and 65 % water (0.1 % formic acid). A liter of each was prepared by adding 1 mL of formic acid (88 % A.C.S. reagent, Aldrich Chemical Company, Milwaukee WI, Catalog #39, 938-8) to each respective solvent.
2. HPLC grade acetonitrile and DI water.
3. USP reference standard, Carisoprodol (Lot F). A stock standard was prepared at approximately 2 mg/mL in acetonitrile. Working standards were prepared by serial dilution with DI water at 40 µg/mL, 20 µg/mL, and 10 µg/mL.

Sample Preparation

Preparation of the carisoprodol tablets consisted of grinding five (5) individual tablets with a mortar and pestle into a fine powder. Each of the individual ground tablets was transferred into an individual 20-mL glass scintillation vial. To each vial, 10 mL of acetonitrile was added and the solutions were sonicated for 15 minutes. A portion of each solution was passed through a 25 mm 0.45 µm nylon syringe filter. Based on internet searches[1], the tablets were suspected of containing 350 mg carisoprodol each; therefore an additional dilution was necessary to decrease the filtrate concentration into a range suitable for MS detection. The filtrate was further diluted by taking a 100 µL aliquot, adding 10 mL DI H_2O, mixing, and then taking 100 µL of this solution to 1 mL with DI H_2O.

Method Validation – LC/MS Assay

Each of two individual tablets was spiked at a different level with a portion of the USP Carisoprodol reference standard. After grinding a single tablet, a known quantity of solid carisoprodol standard equivalent to less than that expected to be present, was added. A second tablet was ground and spiked with a portion of standard in excess of that expected to be present in the tablet. The acetonitrile was added, and the solutions were treated the same as the sample solutions, with the exception of an additional dilution. To bring the spike preparation into the calibration curve range, the final dilution was 30 µL of the spike solution diluted to a total volume of 1 mL with DI water.

Linearity of carisoprodol was established from five separate standards ranging from 0.5 µg/mL to 40 µg/mL. This concentration range was chosen to bracket the diluted sample concentrations. The plot of peak area versus concentration was linear, and the correlation coefficient, r, for carisoprodol was calculated to be 0.9996.

The limit of detection (based on signal:noise of 10:1) was determined for carisoprodol by analysis of a low level standard. The noise level was calculated from the average of ten blank injections, using the area response within the retention time window corresponding to carisoprodol. The detection limit for carisoprodol, on column, was 6.7 picograms.

LC/MS System

The electrospray interface was operated in positive ion scan mode with a mass range of 90-350 amu. The internal capillary voltage was set at 3000 volts. The nitrogen drying gas flow rate used was 10 L/min at 300^{O} C, and the nebulizer pressure was set at 20 psig. For the initial screening, the MS was also operated in the full scan mode with a mass range of 90-350 amu. For the determination of carisoprodol content, the instrument was switched to selected ion monitoring (SIM) mode for more sensitivity and better peak shape. Conditions were set to monitor the protonated molecular ion at m/z 261 $[M + H]^{+}$.

The mobile phase consisted of 35 % acetonitrile and 65 % DI water (both with 0.1 % formic acid), pumped through a C_{18} column at 0.4mL/min. The column thermostat was set at 25^{O} C, the run time was 8 minutes, and 1.0 µL injections were made for all samples and standards.

Data Treatment

Total ion chromatograms were generated for all samples, spikes, standards, and blanks. Each chromatogram was integrated at the retention time corresponding to the retention time of the peak observed in the carisoprodol standard. Peaks that were observed in the blank chromatograms in the retention time range of the carisoprodol peak were small enough that their contribution to the sample peak area was considered negligible. Quantitation of carisoprodol was performed using the data obtained from the SIM ion chromatograms.

Results and Discussion

Initial method development for this work included use of methanol (0.1 % formic acid) in place of acetonitrile in the mobile phase, as well as use of UV detection. However, acetonitrile was chosen for the organic component of the mobile phase because it resulted in increased retention and improved peak shape for carisoprodol. Ideally, carisoprodol would generate an adequate UV signal for determining the carisoprodol content. Based on its structure, it is not expected to absorb at 280 nm or at 214 nm. UV experiments verified no absorbance from this compound, even at 20 times the injection volume used for MS detection. Therefore, quantitation was performed based on SIM data generated by the MS.

Spray chamber parameters were optimized for carisoprodol using the flow injection analysis mode of the instrument. In-source collision induced dissociation (CID) generated fragment ions and was accomplished by adjusting the instrument's fragmentor voltage. Optimum CID conditions were obtained by injecting a 20 µg/mL

carisoprodol standard at several fragmentor voltages followed by review of the resulting mass spectra. In general, higher fragmentor voltage helps the transmission of ions through the relatively high-pressure region between the exit of the capillary and the entrance of the skimmer[4]. At voltages of 30 V or less, very little fragmentation was observed. At voltages of 50 V or greater, excessive fragmentation occurred and there was very little signal observed at $[M + H]^+$ (m/z = 261). At voltages greater than 100 V, neither the protonated molecular ion nor the potassium adduct, $[M + K]^+$ (m/z = 299), was observed in the mass spectrum. A fragmentor voltage of 40 volts was chosen for this analysis because it allowed the detection of structurally useful fragment ions while maintaining sufficient response for the molecular ion.

For screening the samples, full scan MS data was used. Figure 3 depicts a total ion chromatogram for one of the injections of the mid-range standard, and figure 4 shows the corresponding mass spectrum for the carisoprodol peak.

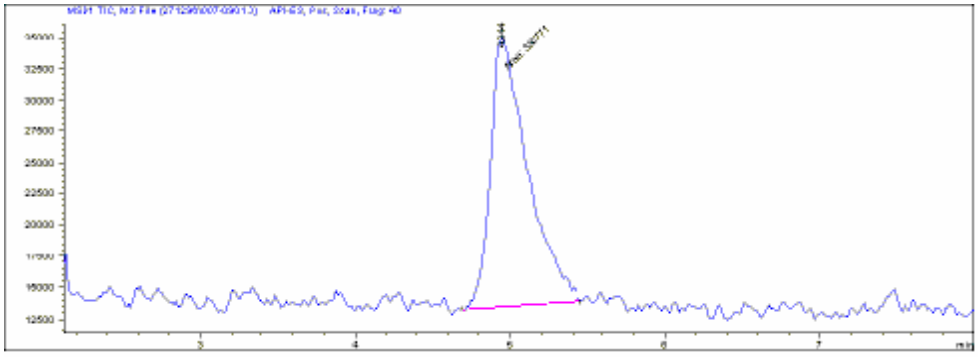

Figure 3: Full-Scan Total Ion Chromatogram for 20µg/mL Carisoprodol Standard

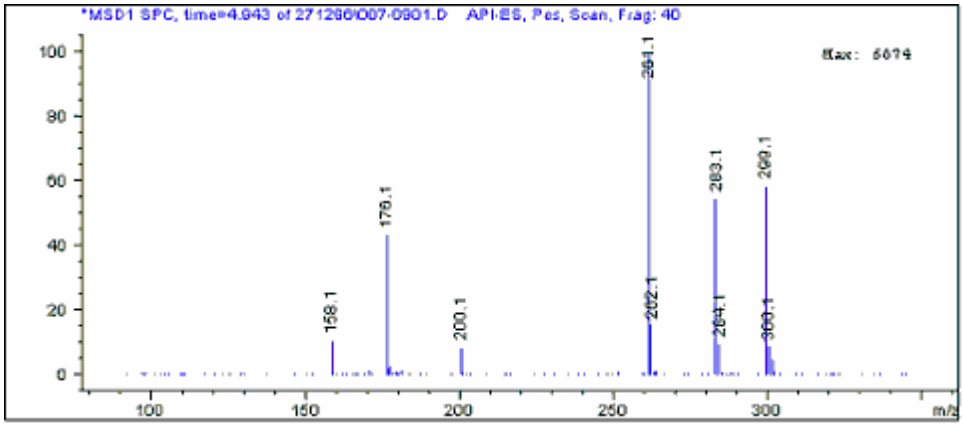

Figure 4: Mass Spectrum of 4.9 min peak in 20µg/mL Carisoprodol Standard Chromatogram

Several of the ions observed in the mass spectrum are considered structurally significant. The ion observed at m/z 261 represents the protonated molecular ion $[M + H]^+$, and the ion observed at m/z 299 represents a potassium adduct $[M + K]^+$. The fragment at m/z 200 is indicative of a loss of a carbamate ion. The likely source of the m/z 158 fragment is through a McLafferty rearrangement of the m/z 200 fragment, and subsequent loss of an isopropyl group. The fragment at m/z 176 is representative of a loss of isopropylformamide from the molecular ion. Figure 5 illustrates the proposed fragmentation pathways.

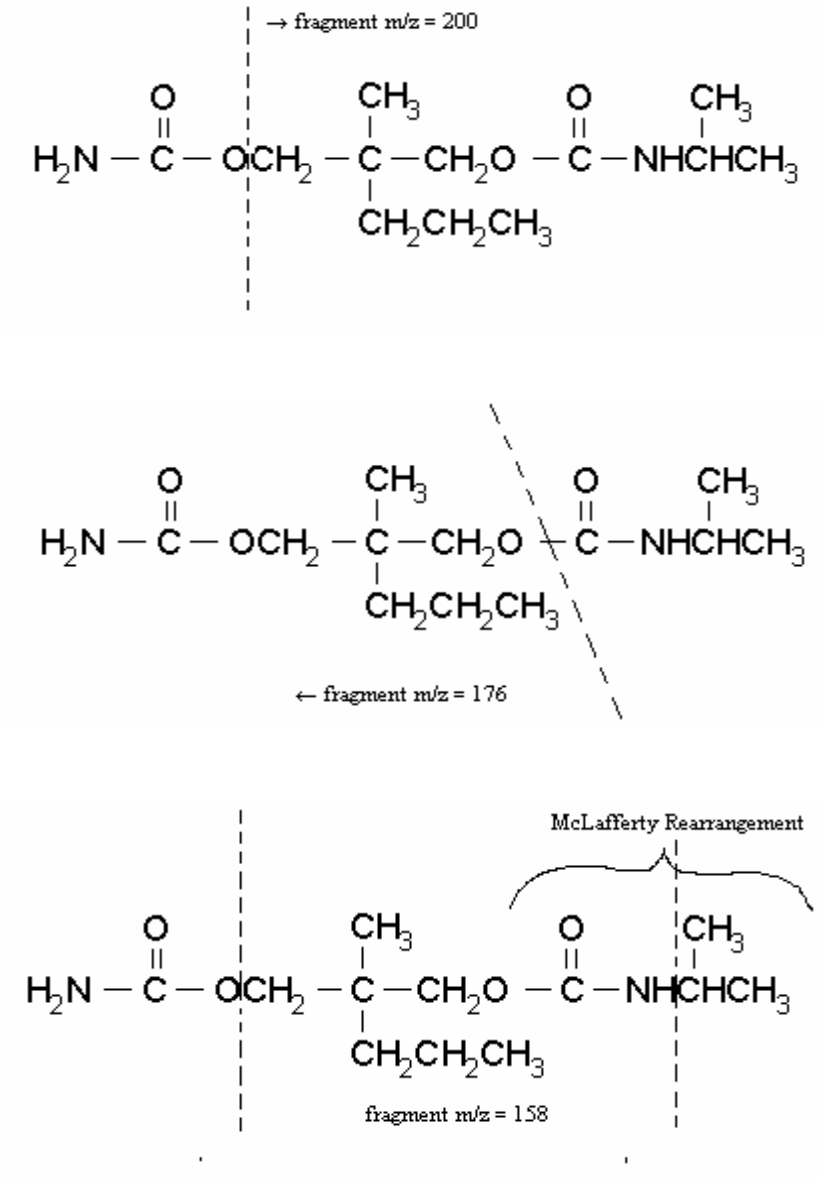

Figure 5: Proposed Fragmentation Patterns for Carisoprodol by ESI-LC-MS

For the determination of the carisoprodol content in the tablets, the SIM data was used. The protonated molecular ion (m/z = 261) was monitored in the SIM experiment. Figure 6 provides an example of a carisoprodol SIM chromatogram. The concentration of carisoprodol present in the tablets was not declared, but based on internet research, the tablets were purported to contain 350 mg carisoprodol each[1]. Five individual tablets were assayed, with results ranging from 322 mg to 329 mg carisoprodol per tablet, and a mean concentration of 325 mg carisoprodol per tablet. This range of values represents an RSD of 1.0 %. Assuming the "declared" value for the tablets is 350 mg, the average value of 325 mg/tablet translates to 93 % of label claim.

Two individual tablets were each spiked with a portion of the USP Carisoprodol reference standard. Tablet 1 was spiked with carisoprodol at a level 0f 554 mg/g, and tablet 2 at a level of 913 mg/g. Recoveries of the carisoprodol were 90 % and 95 %, respectively.

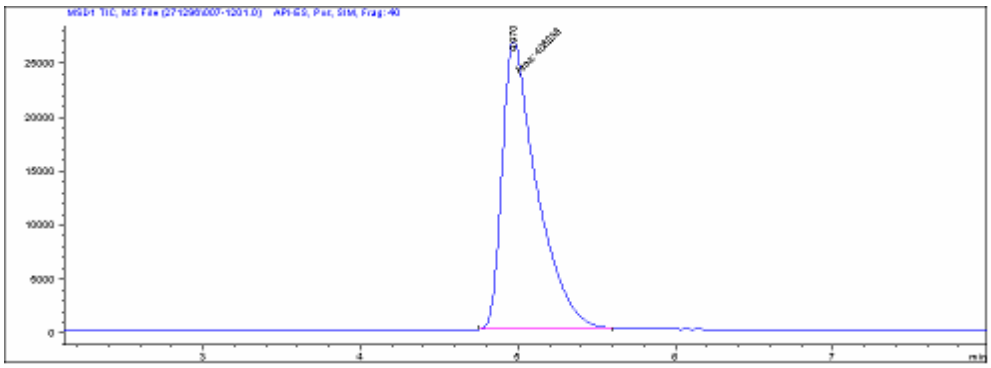

Figure 6: SIM Chromatogram for 20µg/mL Carisoprodol Standard

Conclusions

The analysis of carisoprodol tablets was performed using liquid chromatography with an electrospray interface and mass selective detection. The results obtained for five individual tablets ranged from 322 mg/tablet to 329 mg/tablet with an RSD of 1 %. The accuracy of the method was demonstrated by spike recoveries of 90 – 95 %. The on column detection limit was determined to be 6.7 picograms for carisoprodol. By using the technique discussed, finished dosage forms can be screened for the presence of carisoprodol and the carisoprodol content can accurately be determined.

Acknowledgements

The authors would like to thank Bryan M. Gamble for his assistance with the proposed fragmentation patterns for carisoprodol.

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